Supplementary figures

Aβ-induced acceleration of Alzheimer-related τ-pathology spreading and its association with prion protein

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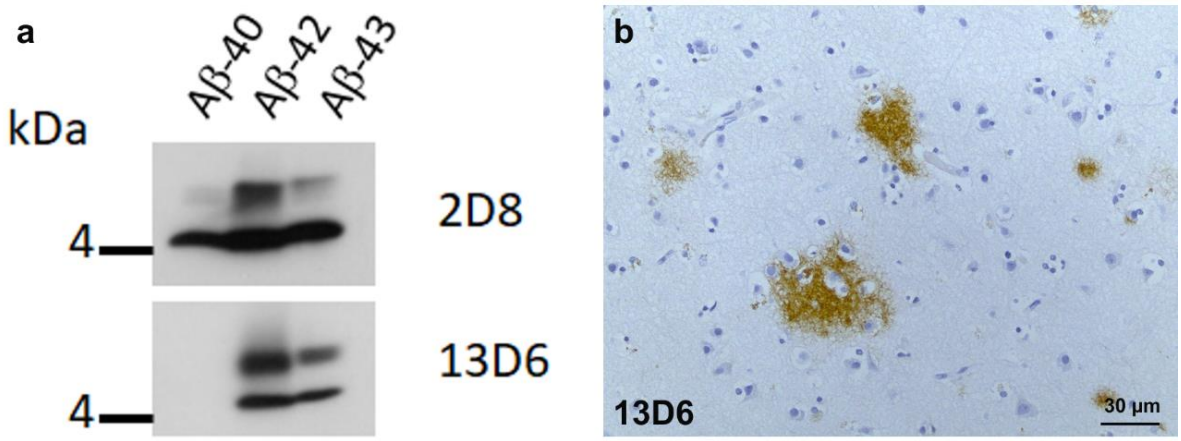


Suppl. Fig. 1: Specificity of the rat monoclonal antibody 13D6 for $A\beta_{42}$ and $A\beta_{43}$. a: 1 ng of each synthetic peptide (JPT Peptide Technologies, Germany) was separated on 10-20% Tris-Tricine gels (Invitrogen, Germany) and transferred on Nitrocellulose membranes. Detection of all three peptides with the N-terminal antibody 2D8 [4] is shown in the upper panel. Detection of the two longer Aβ species, $A\beta_{42}$ and $A\beta_{43,}$ by 13D6 is shown below, while $A\beta_{40}$ remains unrecognized. **b**: Immunohistochemical staining of amyloid plaques with the 13D6 antibody in case No. 15 to prove the detection of $A\beta_{42/43}$ species in plaques with this antibody.

a Percentage of p-τ-positive neurons = $\frac{\text{Number of p-τ-positive neurons x 100}}{\text{Number of total neurons of the reference area}}$

b Percentage of PrP^C+p-τ-positive neurons = $\frac{\text{Number of } PrP^C\text{+p-τ-positive neurons x 100}}{\text{Number of DAPI neurons of the reference area}}$

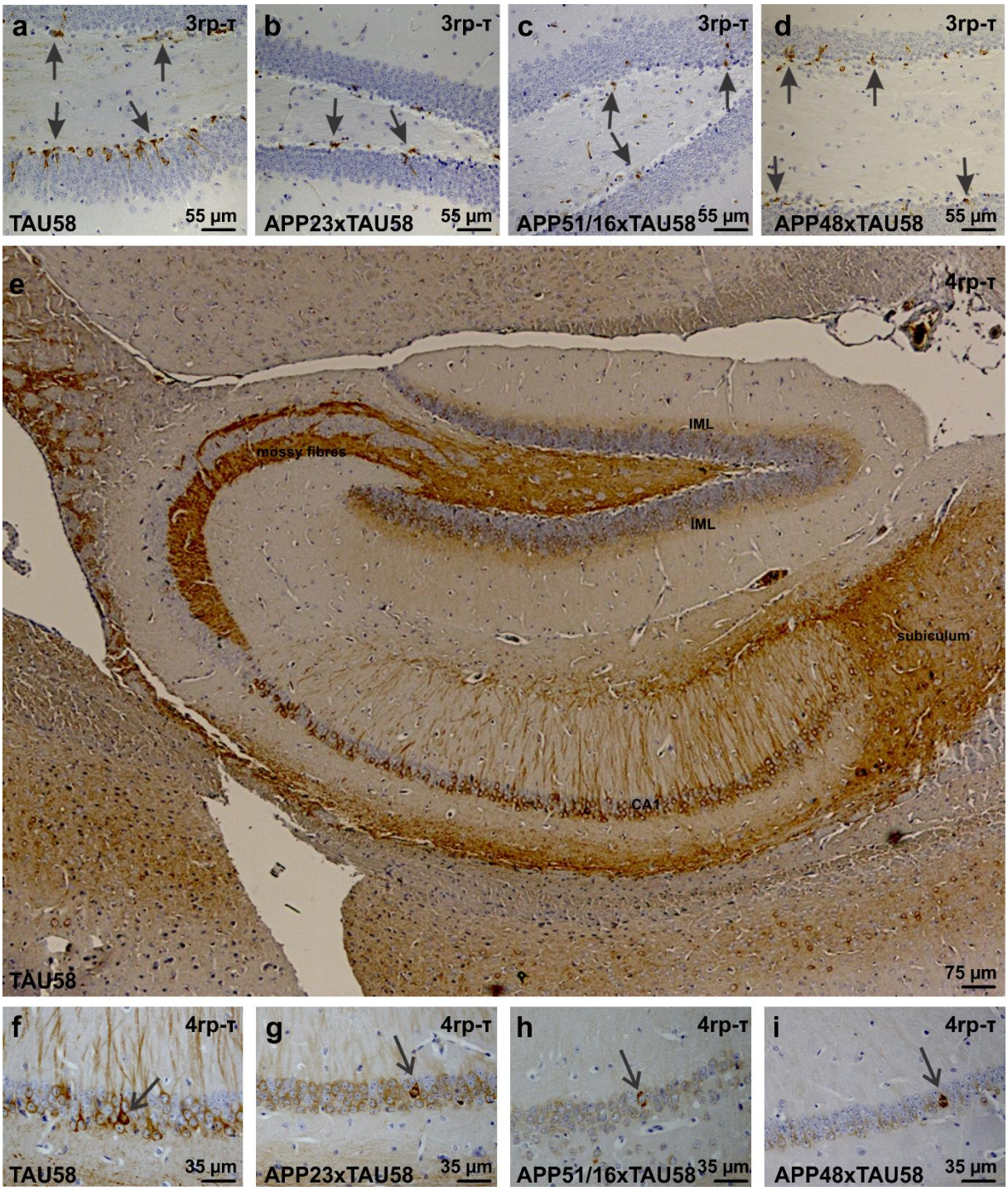
c Percentage of PrP^C+Aβ-positive neurons = $\frac{\text{Number of } PrP^C\text{+Aβ-positive neurons x 100}}{\text{Number of DAPI neurons of the reference area}}$

d Percentage of PrP^C-positive neurons = $\frac{\text{Number of } PrP^C\text{-positive neurons x 100}}{\text{Number of total neurons of the reference area}}$

e Aβ plaque load = $\frac{\text{Area of Aβ-positive plaques x 100}}{\text{Reference area}}$

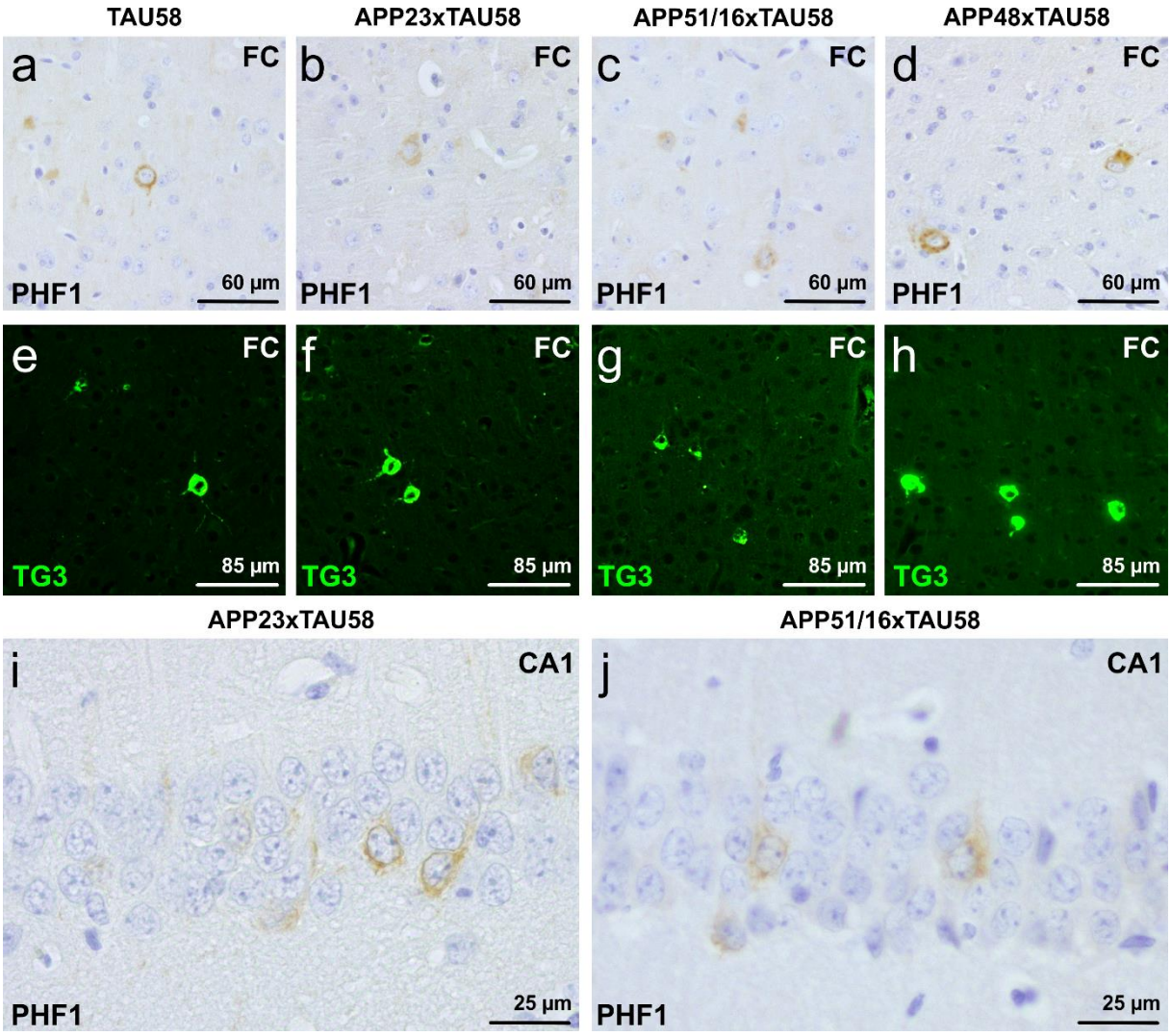
f PrP^C plaque load = $\frac{\text{Area of } PrP^C\text{-positive plaques x 100}}{\text{Reference area}}$

Suppl. Fig. 2: Formulas for the calculation of the percentages of (**a**) p-τ-positive neurons, (**b**) PrP^C+p-τ-positive neurons, (**c**) PrP^C+Aβ-positive neurons, (**d**) PrP^C-positive neurons, and (**e**) the Aβ and (**f**) PrP^C-plaque loads.



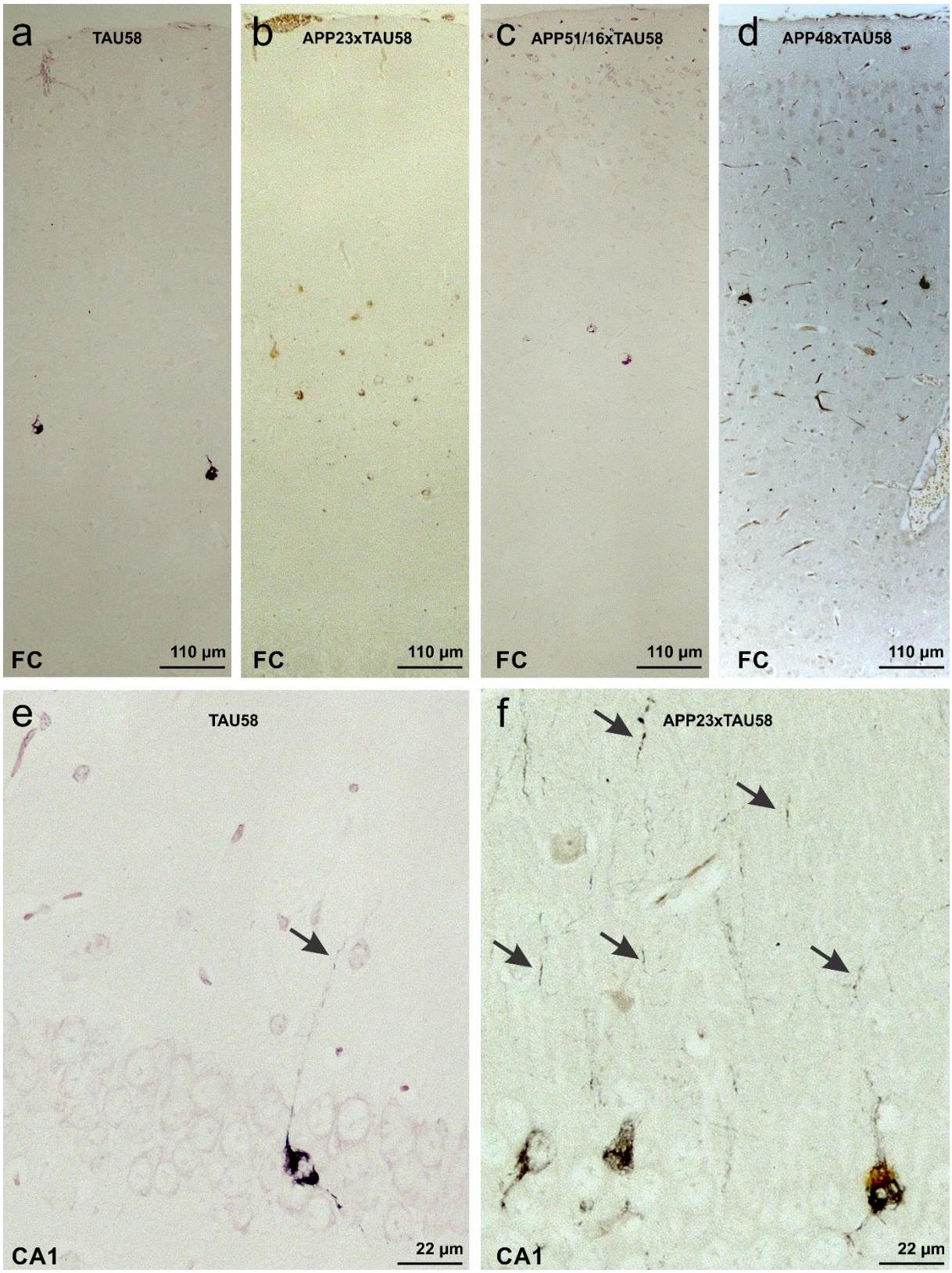
Suppl. Fig. 3: 3 repeat and 4 repeat τ expression in the hippocampus of TAU58, APP23xTAU58, APP51/16xTAU58, and APP48xTAU58 mice. 3 repeat (3rp-τ; **a-d**) and 4 repeat τ (4rp-τ; **e-i**) expression in the hippocampus of TAU58 (**a,e,f**), APP23xTAU58 (**b,g**), APP51/16xTAU58 (**c,h**), and APP48xTAU58 mice (**d,i**) detected immunohistochemically with the antibodies RD3 (3rp-τ) and RD4 (4rp-τ). 3rp-τ expression in the 6-month-old mice of each

genotype was restricted to cells of the subgranular zone (arrows) of the dentate gyrus according to the stage of brain development, and did not vary much among the genotypes of the mice (**a-d**). 4rp-τ expression was observed in the mossy fibers, in CA1 pyramidal cells, in the neurons and the neuropil of the subiculum, and in the neuropil of the inner molecular layer of the dentate gyrus (IML) (**e**). In the hippocampal CA1 sector, nearly all neurons express 4rp-τ but only few neurons in each mouse line exhibit NFT-like condensation of 4rp-τ in their cytoplasm (arrows), without major differences among the genotypes of the τ-transgenic and τ-APP-double transgenic mice (**f-i**).



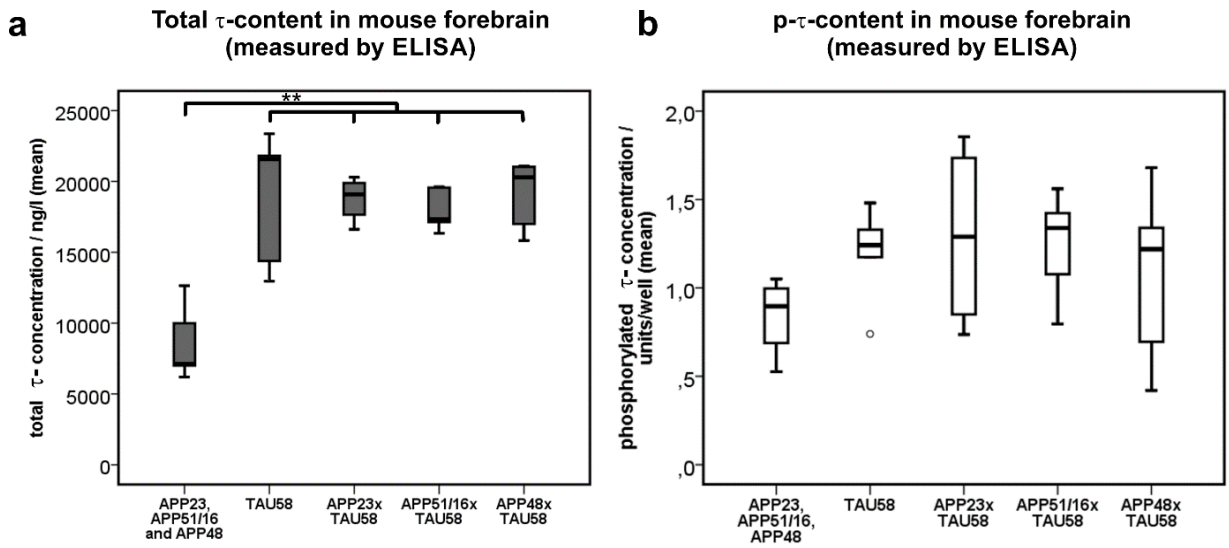
Suppl. Fig. 4: Expression of pathological phosphorylation sites at Ser396/Ser404 and a conformational epitope related to phosphorylated Thr231 of τ (TG3 epitope) in the frontal cortex of TAU58, APP23xTAU58, APP51/16xTAU58, and APP48xTAU58 mice. Expression of pathological phosphorylation sites at Ser396/Ser404 (detected with the PHF-1 antibody; **a-d,i,j**) and a conformational epitope related to phosphorylated Thr231 [2] (detected with the TG3 antibody; **e-h**) of τ in the frontal cortex of TAU58 (**a,e**), APP23xTAU58 (**b,f**), APP51/16xTAU58 (**c,g**), and APP48xTAU58 mice (**d,h**). In the frontocentral cortex (**a-h**), both τ epitopes are expressed in NFT or pretangle-bearing neurons in all types of the above-mentioned transgenic mice. In the CA1 sector of APP23xTAU58 (**i**) and APP51/16xTAU58

mice (**j**), a number of CA1 pyramidal neurons exhibited the phosphorylation epitope of Ser396/Ser404 confirming the presence of p-τ in this region. In all transgenic mouse lines, p-τ-positive neurons were also stained with the AT8 antibody detecting p-τ phosphorylated at Ser202/Thr205 (see Fig. 1).



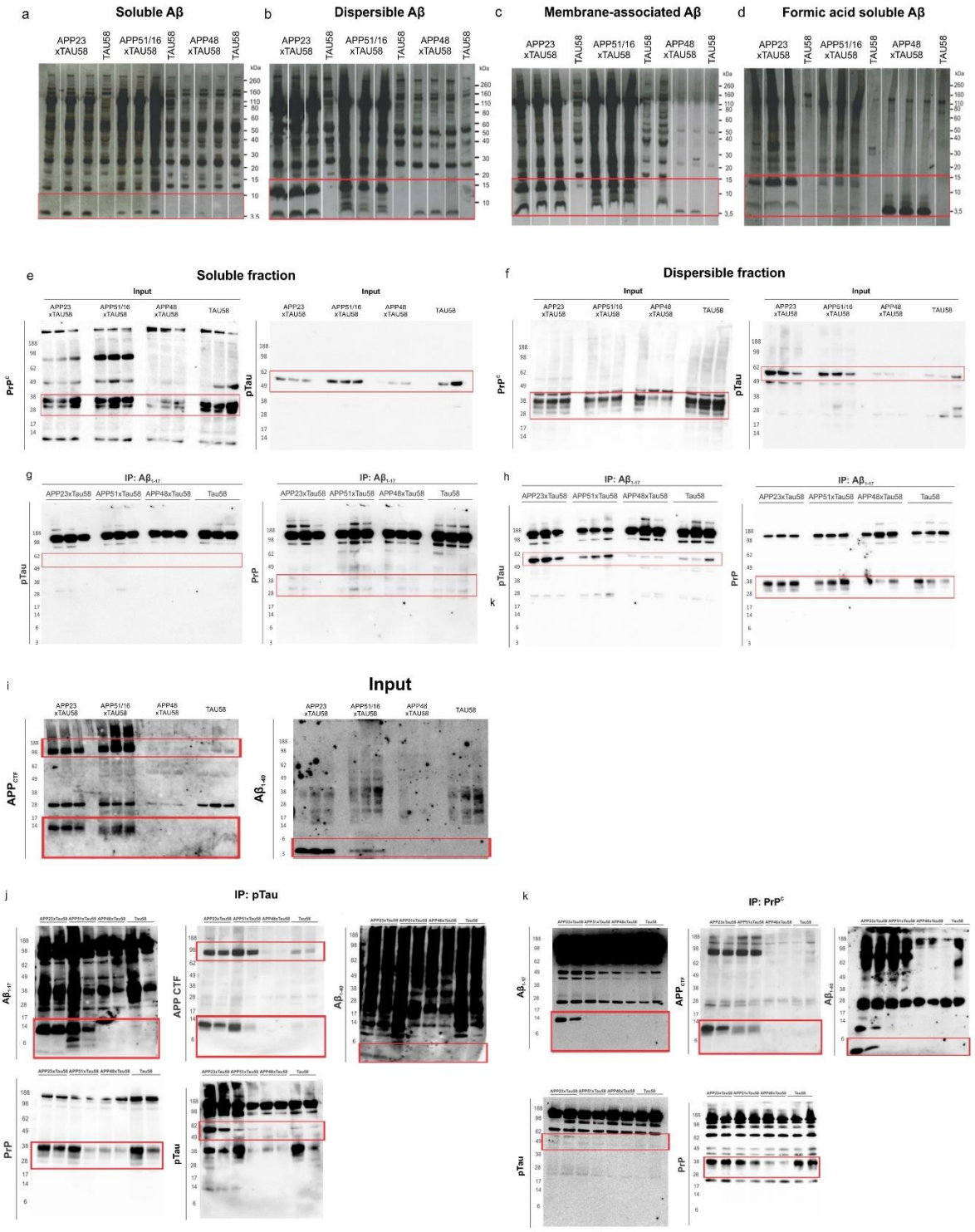
Suppl. Fig. 5: **Detection of argyrophilic NFTs and neuropil threads in TAU58, APP23xTAU58, APP51/16xTAU58, and APP48xTAU58 mice.** Detection of argyrophilic

NFTs and neuropil threads as detected with the Gallyas silver method in TAU58 (**a, e**), APP23xTAU58 (**b, f**), APP51/16xTAU58 (**c**), and APP48xTAU58 mice (**d**) in the frontocentral cortex (**a-d**) and the CA1 sector of the hippocampus (**e, f**). In the frontocentral cortex and in the hippocampal sector CA1, all of the above-mentioned transgenic mouse lines exhibited NFTs and neuropil threads. However, APP23xTAU58 mice showed more Gallyas-positive neurons than TAU58 mice (**e,f**). The neuropil threads (arrows) are best visible in **e** and **f** taken at higher magnification.



Suppl. Fig. 6: **τ ELISA measurements in APP23, APP51/16, APP48, TAU58, APP23xTAU58, APP51/16xTAU58, and APP48xTAU58 mice.** Concentration of total τ (**a**) and p-τ protein (**b**) in forebrain homogenates from APP23, APP51/16, APP48, TAU58, APP23xTAU58, APP51/16xTAU58 and APP48xTAU58 mice as measured by MSD multi-spot phospho (Thr231)/total τ ELISA. As expected, the total τ-concentrations where higher in mice with transgenic overexpression of the τ-protein, but among the different double transgenic mice no significant differences were obvious compared to TAU58 mice (**a**). Similarly, no differences among the double transgenic mouse lines compared to TAU58 mice were observed for the p-τ-concentrations (**b**). Note: here we did not even see a significant difference between TAU58 transgenic mouse lines and τ-wild-type APP-transgenic mice, (* $p < 0.05$, *** $p < 0.001$). The ranges vary because of a sample size of 4-6 animals per group. Mean values and ranges: (**a**) total τ: APP23, APP51/16, APP48 – mean = 8594 ng/l, range = 6193 – 12649 (n = 5); TAU58 – mean = 19275 ng/l, range = 12961 – 23361 ng/l (n = 6); APP23xTAU58 – mean = 18770 ng/l, range = 16623 – 20289 ng/l (n = 4); APP48xTAU58 – mean = 19258 ng/l, range = 15835 – 21093 ng/l (n = 6); APP51/16xTAU58 – mean = 17892 ng/l, range = 16344 – 19622 ng/l (n

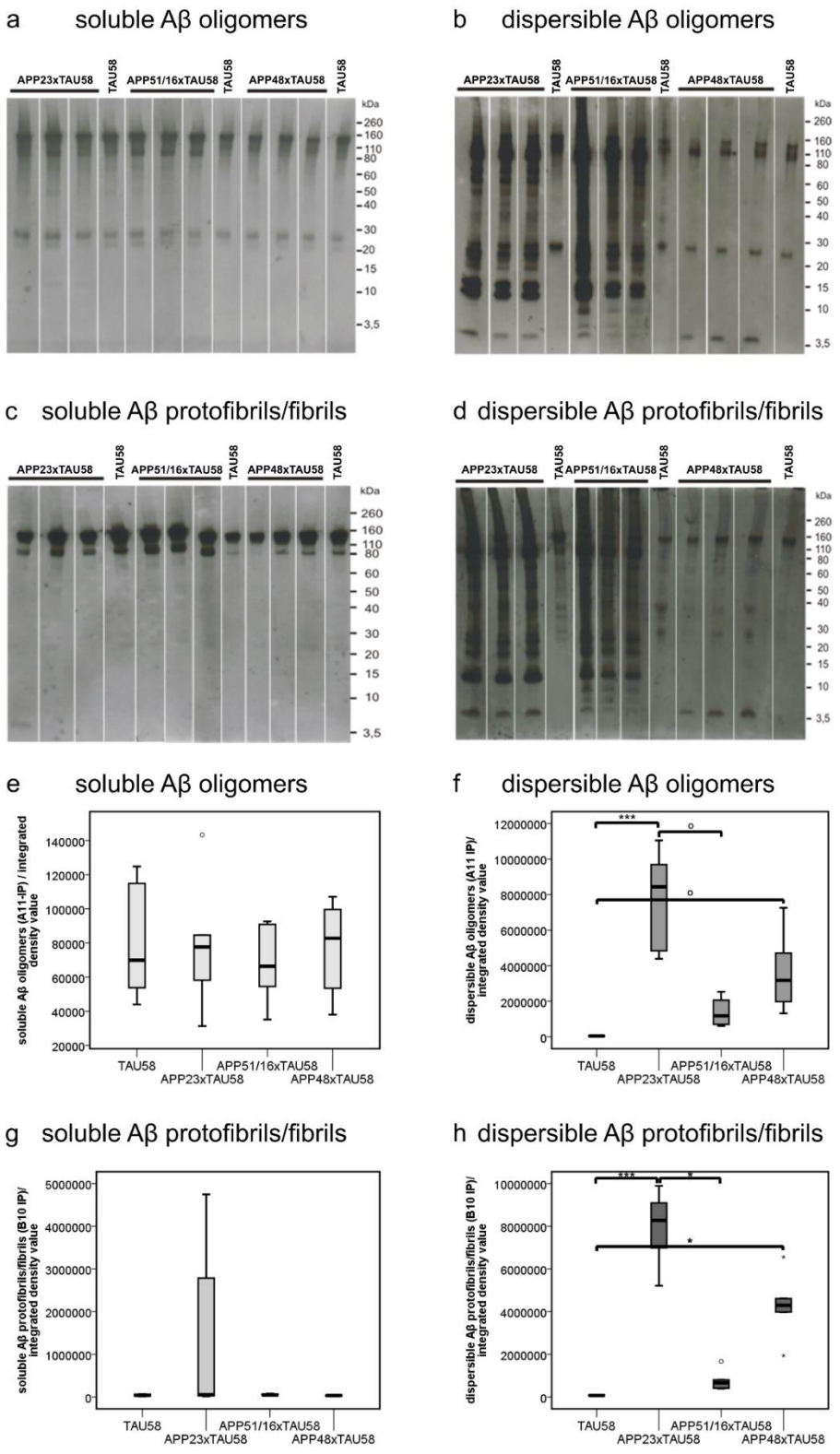
= 6). (**b**) p-τ: APP23, APP51/16, APP48 – mean = 0.84 units/well, range = 0.53 – 1.05 unit/well (n=4); TAU58 – mean = 1.20 units/well, range = 0.74 – 1.48 units/well (n = 6); APP23xTAU58 – mean = 1.29 units/well, range = 0.74 – 1.85 units/well (n = 4); APP48xTAU58 – mean = 1.10 units/well, range = 0.42 – 1.68 units/well (n = 6); APP51/16xTAU58 – mean = 1.26 units/well, range = 0.80 – 1.56 units/well (n = 6).



Suppl. Fig. 7: Uncropped blots with molecular weight standard from Figs. 2 and 3. Western blot analysis of soluble (**a**), dispersible (**b**), membrane-associated (**c**) and formic acid-soluble Aβ (**d**) in forebrain homogenates from APP23xTAU58, APP51/16xTAU58, APP48xTAU58 and TAU58 mice detecting with 6E10 antibody against $A\beta_{1\text{-}17}$. Aβ molecular

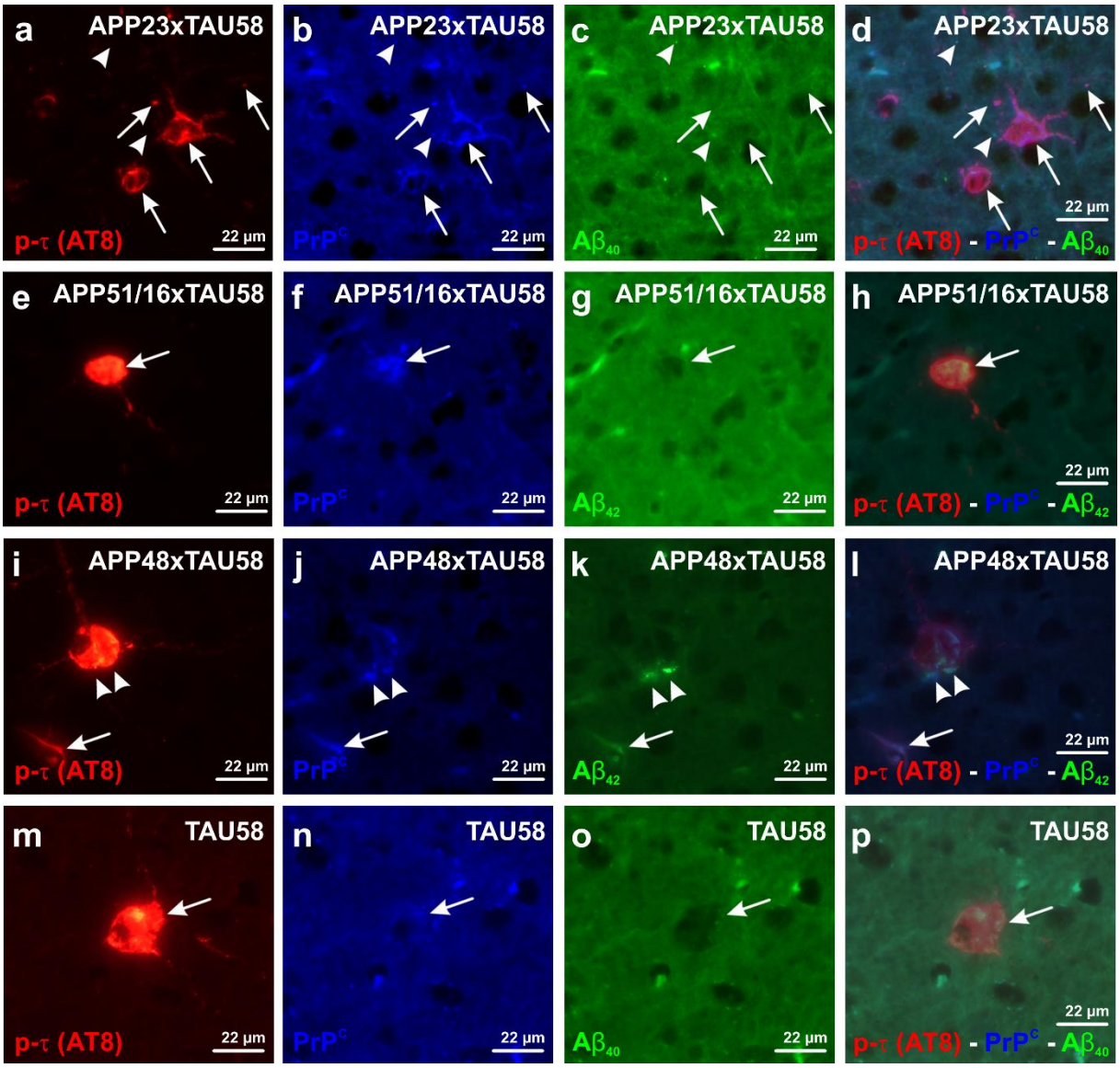
weight ~4 kDa. **e,f:** Western blot analysis of PrP^C (6D11) and p-τ (PHF1) input levels in soluble and dispersible fractions. **g,h:** Immunoprecipitation with subsequent western blot analysis of soluble and dispersible fractions precipitated with the anti-$A\beta_{1-17}$ antibody (6E10) and detected with antibodies against PrP^C (6D11) and p-τ (PHF1) (**g**) in APP23xTAU58, APP51/16xTAU58, APP48xTAU58 and TAU58 mice. Neither PrP^C (33-35 kDa) nor p-τ (45-69 kDa [1]) were linked to $A\beta_{1-17}$ in the soluble fraction (**g**). PHF1 detection was performed after stripping of PrP^C antibody. In the dispersible fraction, PrP^C and p-τ were shown to co-immunoprecipitate with $A\beta_{1-17}$, exhibiting stronger signals in the APP23xTAU58 and APP51/16xTAU58 mice when compared to APP48xTAU58 and TAU58 mice (**h**). The molecular weight bands observed at ~62 kDa and higher appeared to represent unspecific bands. **i:** Western blot analysis of APP/APP_{CTF} and $A\beta_{40}$ input levels in the dispersible fraction. **j:** Immunoprecipitation with subsequent western blot analysis of dispersible fractions precipitated with p-τ (PHF1). Anti-$A\beta_{1-17}$ (6E10) detected APP ~98 kDa[3]), $APP_{CTF\beta}$ (~11 kDa[3]) and a very faint band around 4 kDa (Aβ) in APP23xTAU58. In APP51/16xTAU58 mice, $APP_{CTF\alpha}$ and monomeric and dimeric Aβ (~4 and 8 kDa) were detected thereby. Bands around ~35 kDa were attributed to previous staining of the membrane. Detection with APP_{CTF}-specific antibody confirmed that bands around 11 kDa were $APP_{CTF\alpha}$ and $APP_{CTF\beta}$ in APP23xTAU58 and APP51/16xTAU58 mice, respectively. In addition, APP (~98 kDa) was immunoprecipitated by the PHF1 anti-p-τ antibody in APP23xTAU58, APP51/16xTAU58 and, in less extent, in TAU58 mice. $A\beta_{40}$ was specifically detected in APP23xTAU58 mice (~4 kDa). PrP^C (33-35 kDa) was co-immunoprecipitated with p-τ in all the models. As positive control, the detection with anti-p-τ (AT8) revealed immunoprecipitation of p-τ, especially in APP23xTAU58 mice, as expected from the p-τ input levels. Unspecific high molecular weight bands were observed around 98 and 188 kDa. $A\beta_{1-17}$ was detected after stripping the anti-PrP^C incubated blot. In a second blot, $A\beta_{40}$ and APP_{CTF} were detected in the same membrane after respective stripping steps. **k:**

Immunoprecipitation with subsequent western blot analysis of dispersible fractions precipitated with PrP^C (6D11). Anti-$A\beta_{1-17}$ (6E10) only detected an 11 kDa band of $APP_{CTF\beta}$ in APP23xTAU58 mice. When detected with an anti-APP antibody directed against the C-terminus of APP, similar bands were seen in APP23xTAU58 mice, which are attributed to $APP_{CTF\beta}$ (~11 kDa). Additionally, faint $APP_{CTF\alpha}$ bands were observed in APP51/16xTAU58 mice. APP (~98 kDa) was also co-immunoprecipitated with PrP^C in APP23xTAU58 and APP51/16xTAU58 mice. $A\beta_{1-40}$ was specifically detected in APP23xTAU58 mice (~ 4 kDa). Few p-τ (~55 kDa) was co-immunoprecipitated with PrP^C in APP23xTAU58 mice. As positive control, the detection with anti-PrP^C (6D11) revealed immunoprecipitation of PrP^C in all models, with increased levels in APP23xTAU58 and TAU58 mice. Unspecific high molecular weight bands were observed around 98 and 188 kDa. p-τ was detected after stripping the anti-$A\beta_{1-17}$ incubated blot. In a second blot, APP_{CTF} and $A\beta_{40}$ were detected in the same membrane after respective stripping steps. Red rectangles correspond to cropped area shown in Figs. 2 and 3.



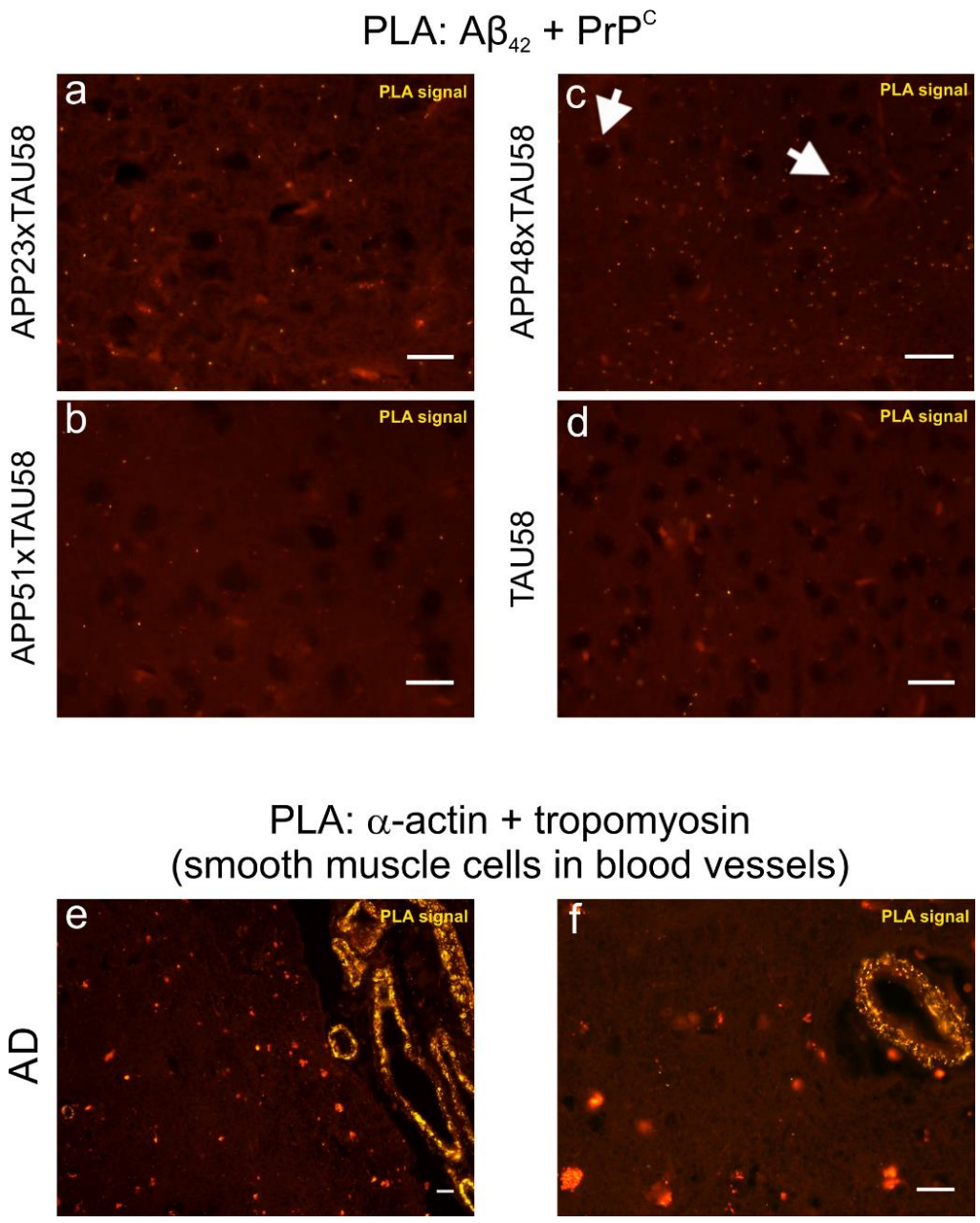
Suppl. Fig. 8: **Detection of soluble and dispersible Aβ oligomers, protofibrils and fibrils in forebrain homogenates from APP23xTAU58, APP51/16xTAU58, APP48xTAU58 and**

TAU58 mice. Immunoprecipitation with subsequent western blot analysis of soluble and dispersible Aβ oligomers precipitated with the A11 antibody (**a,b**) and Aβ fibrils and protofibrils precipitated with B10-AP antibody fragments (**c,d**) in forebrain homogenates from APP23xTAU58, APP51/16xTAU58, APP48xTAU58 and TAU58 mice and its semiquantitative analysis (**e-h**). In none of the mice, soluble Aβ oligomers, protofibrils or fibrils were evident (**a,c,e,g**). Dispersible Aβ oligomers, protofibrils and fibrils were found in APP23xTAU58, APP48xTAU58 and APP51/16xTAU58 mice, with APP23xTAU58 and APP48xTAU58 mice having significantly higher levels than TAU58 mice (**b,d,f,h**). In TAU58 mice, no dispersible Aβ oligomers, protofibrils and fibrils were observed, as expected (**b,d,f,h**). (O p(uncorrected) < 0.05, * p(corrected) < 0.05, *** p(corrected) < 0.001)



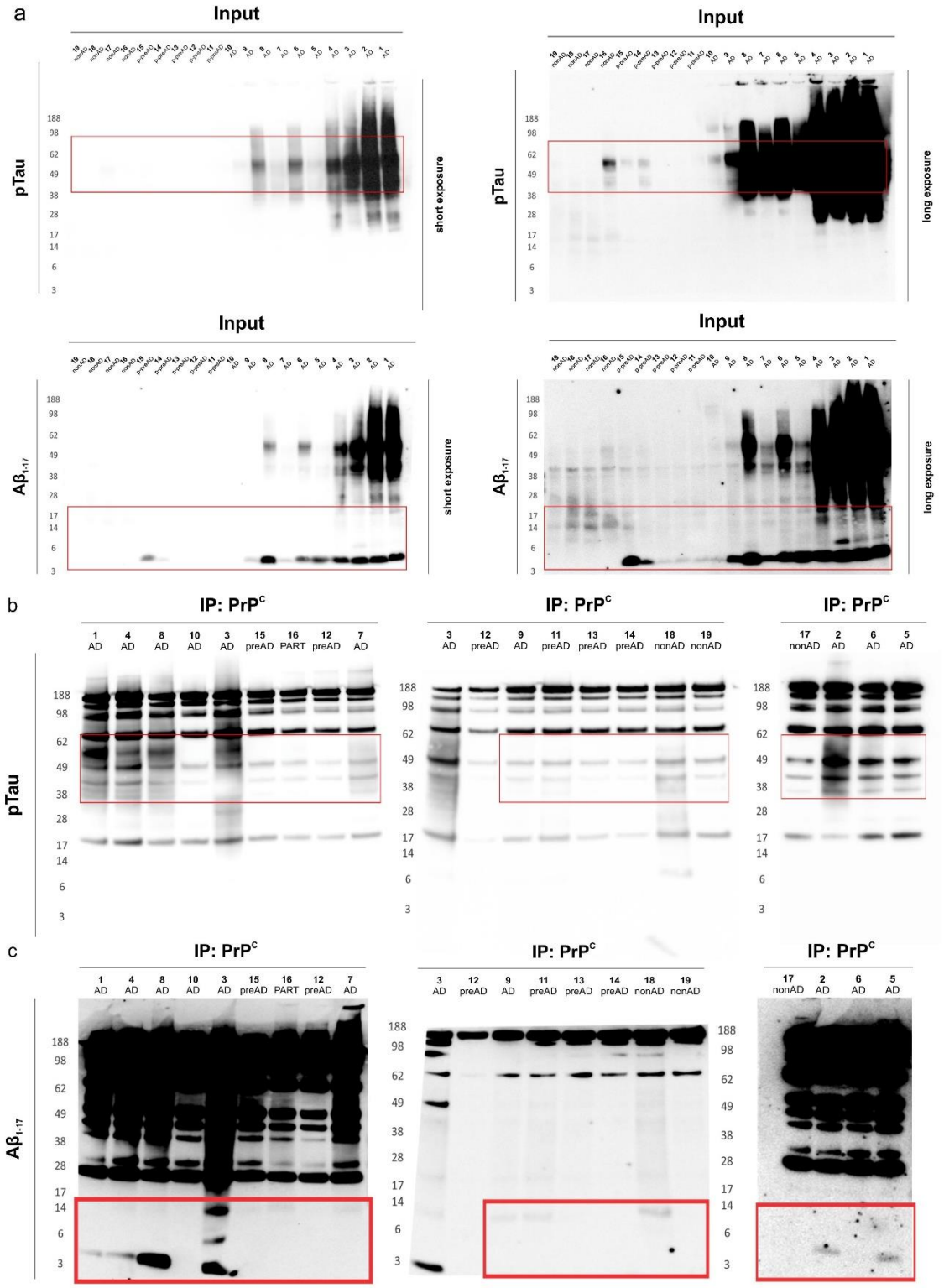
Suppl. Fig. 9: Co-localization of PrP^C, p-τ and Aβ in neurofibrillary tangle-bearing neurons of APP23xTAU58, APP51/16xTAU58, APP48xTAU58, and TAU58 mice. a-p: Representative images of the triple-label immunofluorescence with antibodies raised against p-τ (AT8), PrP^C (6D11), and $Aβ_{40}$ (rabbit polyclonal) or $Aβ_{42}$ (rabbit polyclonal) of the frontocentral cortex of APP23xTAU58 (**a-d**), APP51/16xTAU58 (**e-h**), APP48xTAU58 (**i-l**) and TAU58 (**m-p**) mice. p-τ was detected with the AT8 antibody and labelled in red with Cy5-linked secondary antibodies. PrP^C was detected with the 6D11 antibody labelled with Cy3-linked secondary antibodies and pseudo-coded in blue. Aβ was detected with anti-$Aβ_{40}$ or anti-

$A\beta_{42}$ specific antibodies and visualized in green by labelling with Cy2-linked secondary antibodies. Arrows point to AT8-PrP^C co-localization. In APP23xTAU58 and APP51/16xTAU58 mice, co-localization of PrP^C with p-τ was observed as well as the occurrence of Aβ at neuritic membranes of the same neurons, but usually at different places of the cell, i.e. co-localization of p-τ and PrP^C was seen in the perikaryon (arrows in **a-h**), whereas small dots of $A\beta_{40}$ positivity were found in the neuropil co-localizing with PrP^C (arrowheads in **a-h**). In contrast, APP48xTAU58 mice showed a co-localization of p-τ with Aβ and PrP^C in the perikaryon of single neurons (arrowheads in **i-l**). In TAU58 mice, most neurons contained only τ-positive NFTs, which were not associated with the accumulation of PrP^C (**m-p**; arrow).



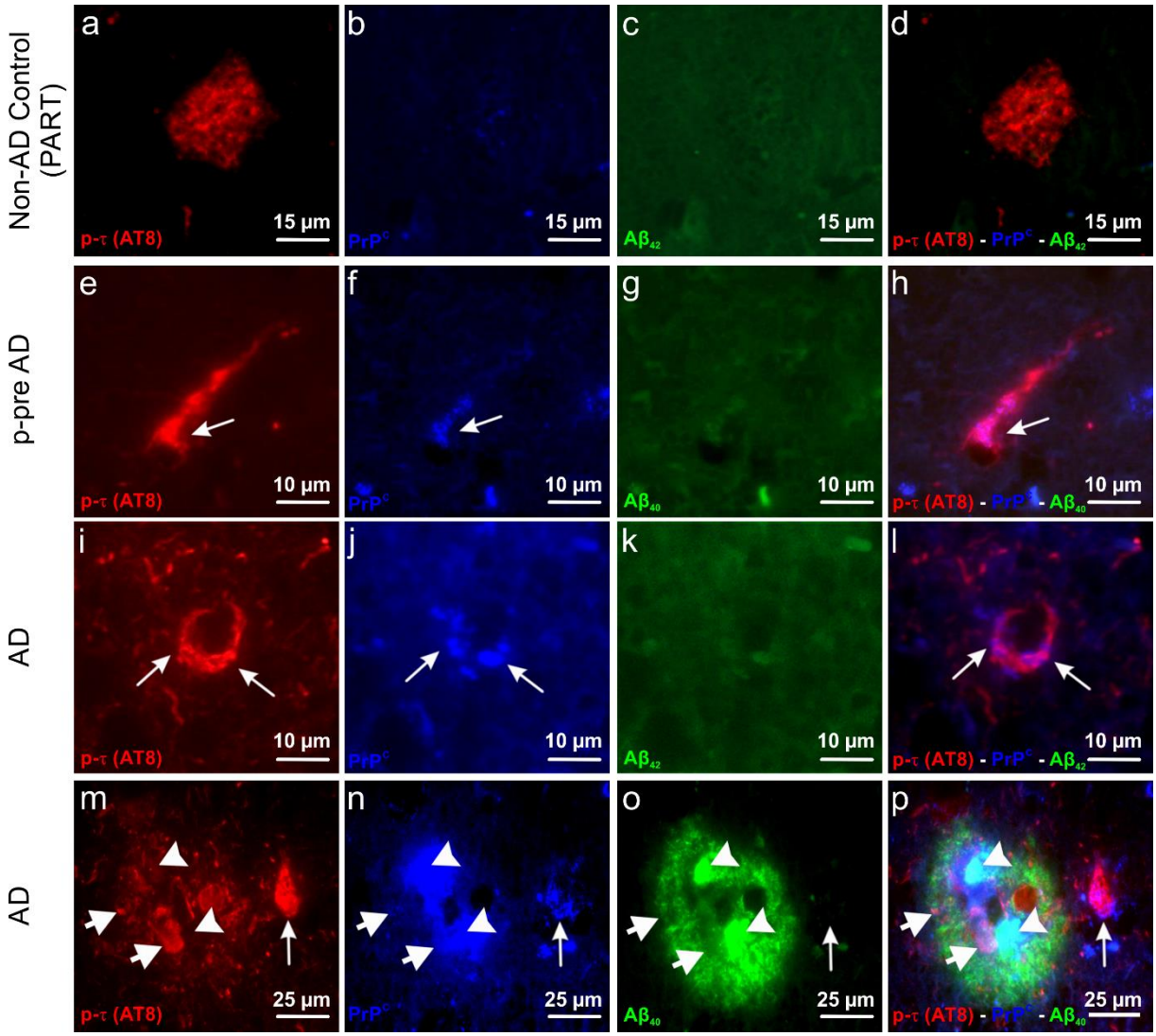
Suppl. Fig. 10: Proximity ligation assays (PLA) between PrP^C and $A\beta_{42}$ in transgenic mice and between alpha-smooth muscle actin (ACTA2) and tropomyosin (α1 isoform) in the human brain. a-d: Representative images of proximity ligation assay using primary antibodies against PrP^C (6D11) and $A\beta_{1\text{-}42}$. PLA signal is observed in every tested transgenic line. In APP51/16xTAU58 and TAU58 mice the PLA signal was seen in a very low (**b, d**), presumably unspecific extent comparable to the random proximity observed in the negative control areas in

e and **f**. In contrast, APP48xTAU58 mice display abundant signal in the neuropil as well as in single neuronal cytoplasmata (**c**: arrows), which is in accordance with their exclusive production of $A\beta_{42}$. The neurons were identified by the unstained nuclear shadow and lipofuscin granules (reddish granules) in the cytoplasm next to the PLA-signals (yellowish). APP23xTAU58 mice also show increased proximity labelling between $A\beta_{42}$ and PrP^C, in the neuropil (a) as depicted in Fig. 5b), and which is higher than in the negative areas of the PLA control in **e** and **f**. **e, f:** As positive and intrinsic negative control for the PLA binding experiments shown in **a-d** and in Fig. 5m-s, a PLA assay using primary antibodies against well-known, physiological interactor proteins, i.e., smooth muscle actin and tropomyosin was performed in brain sections of case No. 8. Smooth muscle actin and tropomyosin are proteins that occur in smooth muscle cells (e.g. in cerebral blood vessels), where they are bound to one another, but they are not present in the brain parenchyma. Here, the proximity between smooth muscle actin and tropomyosin was restricted to the smooth muscle cells in the vessel wall of arteries and veins whereas only very few dots were seen in the brain parenchyma (**f**), presumably reflecting random proximity of the antibodies. This random proximity signal is significantly lower than the one seen in Figs. 5a,b,d,g,j,p-s, confirming the specificity of the PLA indicating proximity between p-τ and PrP^C, Aβ and PrP^C, Aβ and p-τ rather than random proximity of the antibodies.



Suppl. Fig. 11: Uncropped blots with molecular weight standard corresponding to Fig. 6a, b. a: Input levels of $A\beta_{1-17}$ and p-τ in the sarkosyl-insoluble fraction (P2) of entorhinal cortex brain homogenates from nonAD (n=4, including two PART cases), p-preAD (n=5) and

sympAD (n=10) cases. $A\beta_{1\text{-}17}$ was detected after stripping the membranes stained with the PHF-1 antibody against p-τ. To document low levels of p-τ and Aβ, we show long exposure images of the blots in which the signals of the AD cases are overexposed. **b,c**: Immunoprecipitation with the 6D11 antibody against PrP^C and subsequent western blot analysis with antibodies raised against p-τ (PHF-1) or $A\beta_{1\text{-}17}$ (6E10). The PHF1 antibody detected p-τ (~55 kDa) in 7 out of 10 AD after immunoprecipitation with anti-PrP^C. p-preAD and non-AD controls did not exhibit significant levels of co-precipitated p-τ (**b**). Cases No. 1,2,3,4 and 8 showed the highest levels of PrP^C-bound p-τ (the arrow indicates the p-τ band), followed by cases Nos. 6 and 9. The 6E10 antibody detected Aβ monomers (~4 kDa) in 6 out of 10 AD cases (**c**). No PrP^C-bound Aβ was detected in p-preAD or nonAD cases. Cases Nos. 2 and 8 showed stronger bands for monomeric Aβ, with case No. 2 also displaying 8 and 14 kDa bands that can be attributed to dimeric/trimeric forms of Aβ and APP_{CTFs}, respectively. Cases Nos. 1, 3, 4 and 5 showed a weaker interaction with PrP^C. The bands observed at 25 kDa and 50 kDa are considered to represent the immunoprecipitation-typical IgG light-chain and heavy-chain bands. The high molecular bands above 62 kDa are considered to be unspecific. $A\beta_{1\text{-}17}$ was detected after stripping the membranes stained with the PHF-1 antibody against phosphorylated τ-protein. Red rectangles correspond to the cropped areas shown in Fig. 6a, b.



Suppl. Fig. 12: **Triple label immunofluorescence of PrP^C, p-τ and Aβ in neurons and plaques in the human brain of non-AD (PART), p-preAD and AD cases**. Representative images of the triple fluorescence labelling of the hippocampal region of one non-AD (PART) (**a-d**), one p-preAD (**e-h**) and two symptomatic AD (**i-p)** cases. p-τ was detected with the AT8 antibody and labelled in red with Cy5-linked secondary antibodies. PrP^C was detected with the 6D11 antibody labelled with Cy3-linked secondary antibodies and pseudo-coded in blue. Aβ was detected with anti-$Aβ_{40}$ or anti-$Aβ_{42}$ specific antibodies and visualized in green by labelling with Cy2-linked secondary antibodies. In the PART, non-AD case (case No. 16), NFTs were seen in the subiculum and did not show significant co-localization with PrP^C or Aβ (**a-d**). In the

transentorhinal region of p-preAD case No. 14, there was a p-τ-positive neuron co-expressing PrP^C (long arrow in **e-h**). Such a co-localization of p-τ and PrP^C was also seen in single neurons of the temporal neocortex (Brodmann area 36) of AD case No. 1 (long arrows in **i-l**). A cored plaque in the temporal cortex (Brodmann area 36) of case No. 8 showed co-labelling of Aβ and PrP^C, especially in the plaque core region (arrowheads in **m-p**) whereas single dystrophic neurites exhibited p-τ, PrP^C, and Aβ in the same neurites (short arrows in **m-p**). Near the plaque is a NFT, which showed co-expression of p-τ and PrP^C (long arrow in **m-p**).

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