Binding of metal ion induced tau oligomers to lipid surfaces is enhanced by GSK-3β mediated phosphorylation.

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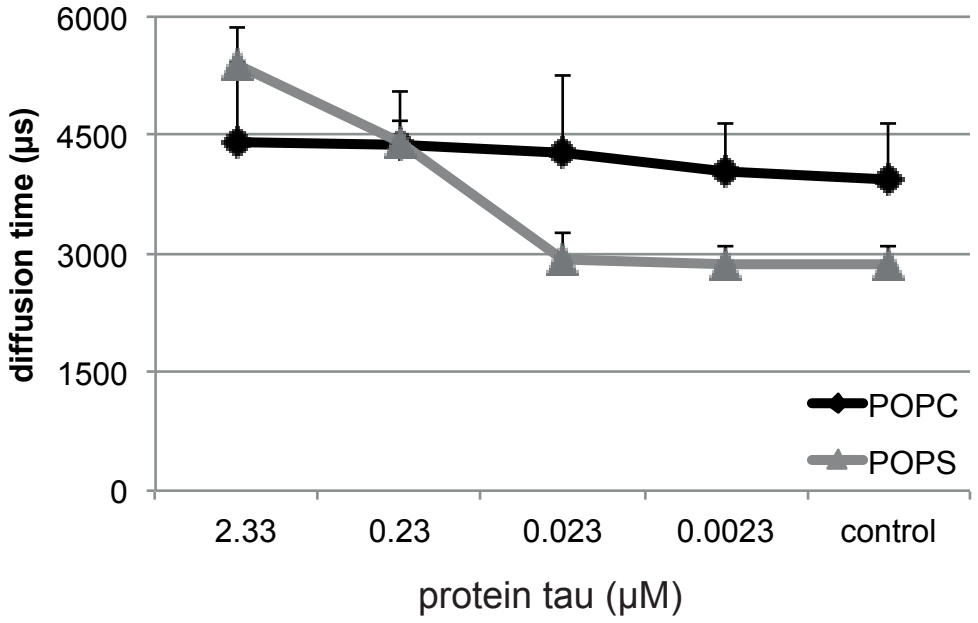
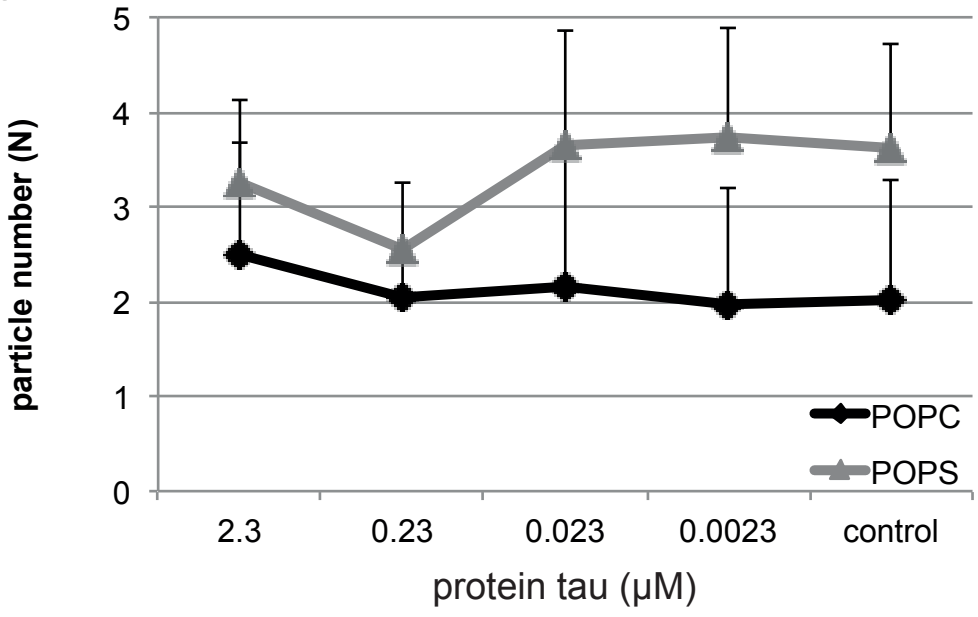
Supplement figures S1- S5

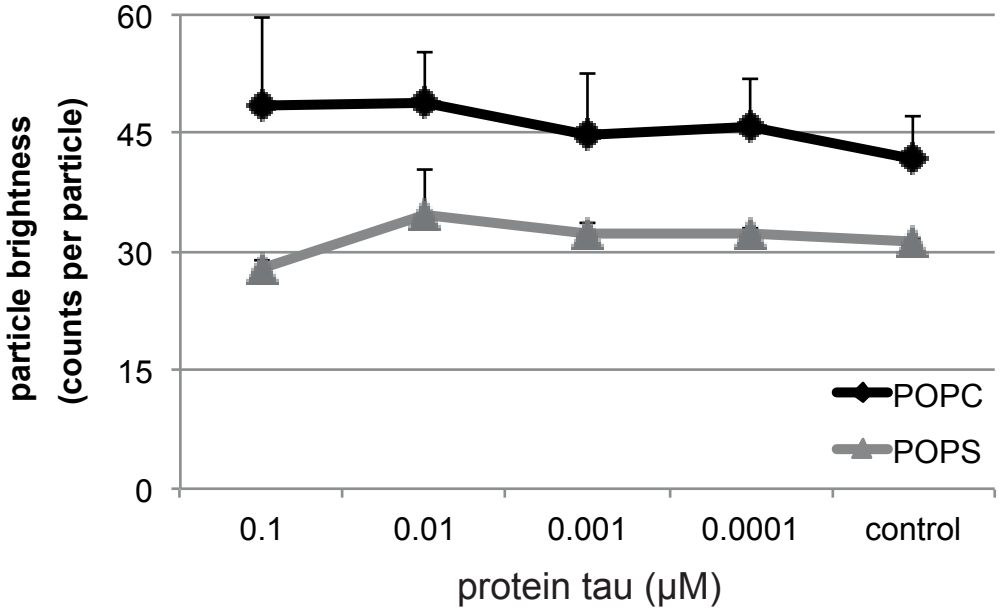
a.



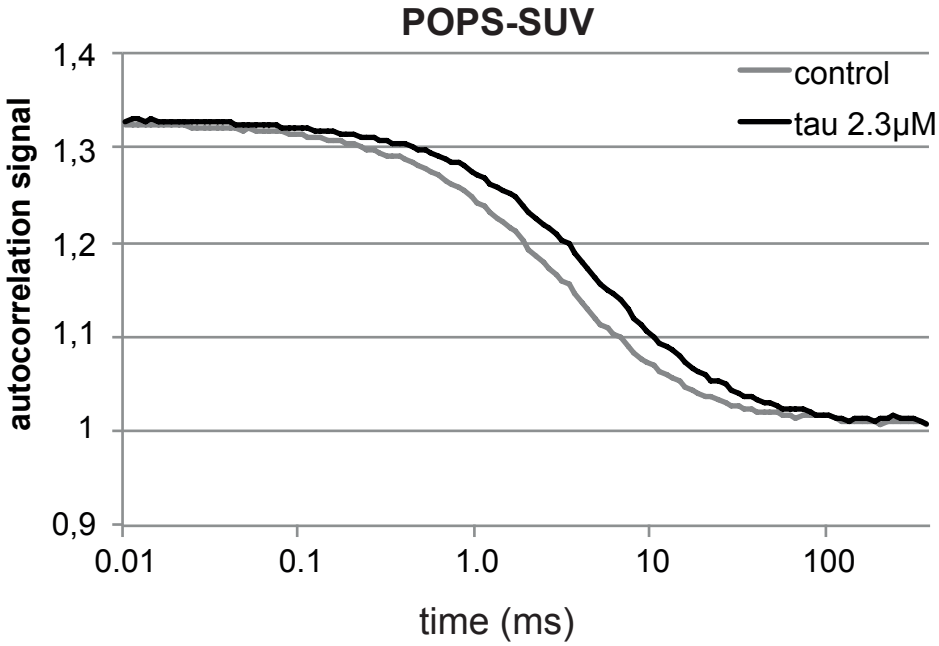
b.



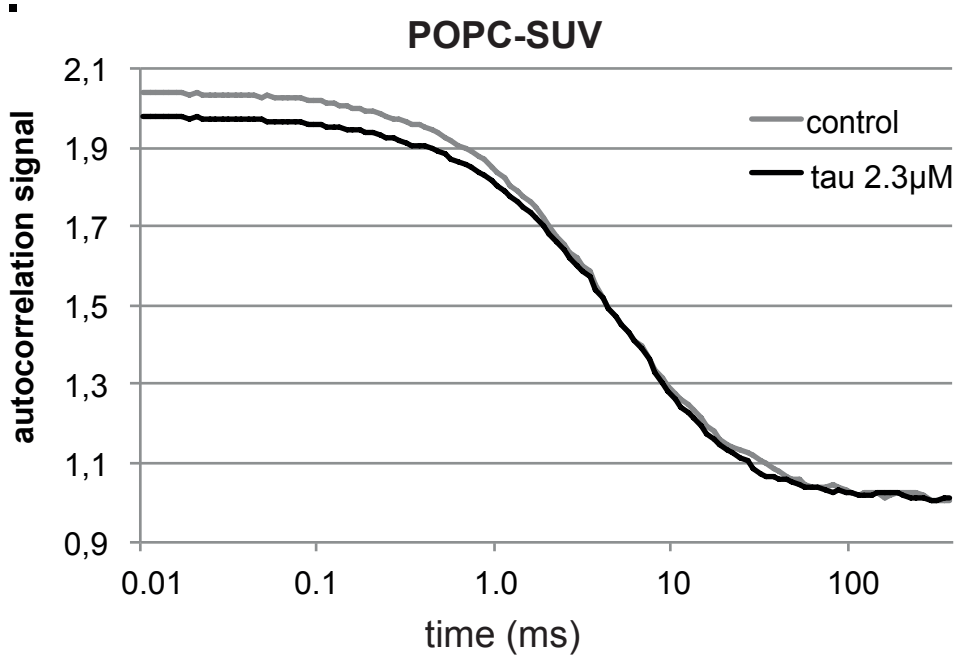
c.



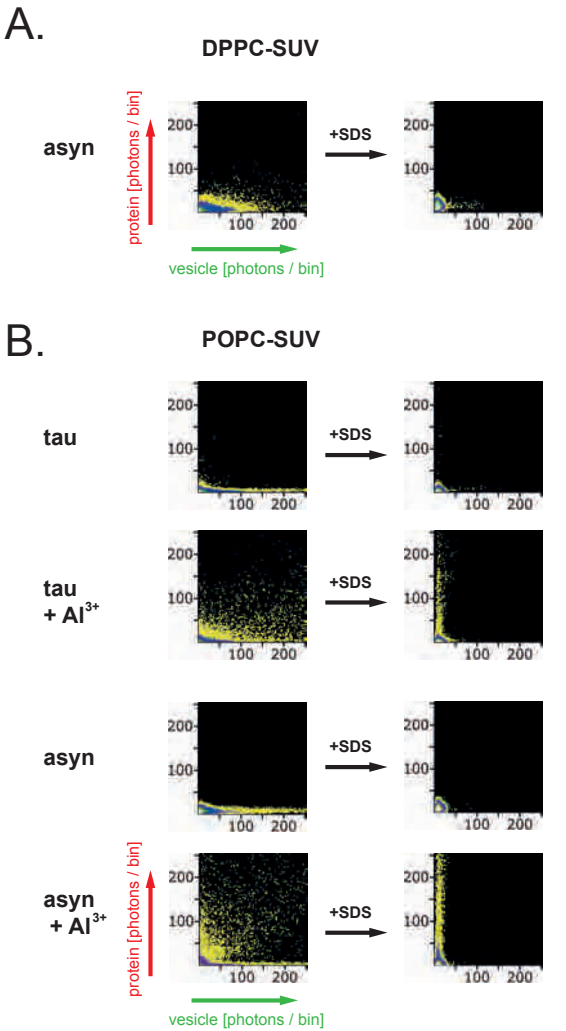
d.



e.



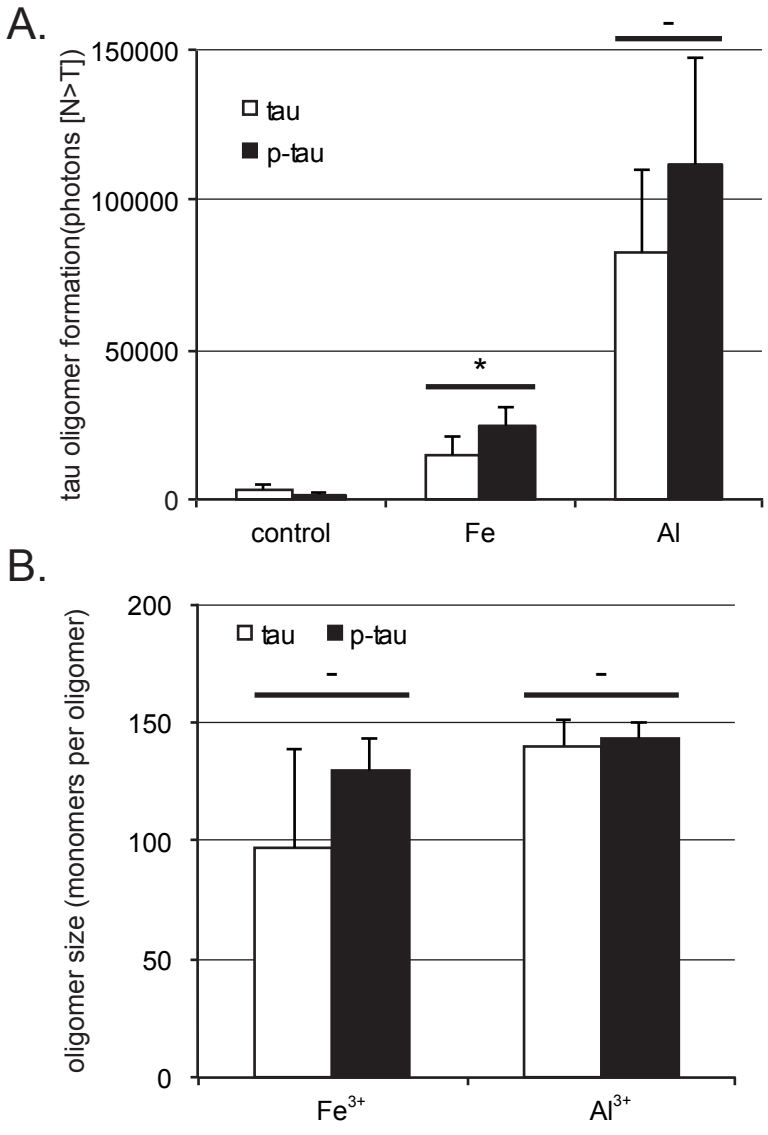
Supplement figure S1
a. FCS measurements of POPC-SUV and POPS-SUV demonstrate an increase in diffusion time for POPS-SUV exposed to high concentrations of unlabeled protein tau, indicating protein binding to lipid vesicles. Since interactions of tau and POPS-SUV were not detected at protein concentrations suitable for SIFT (~10 nM), no SIFT experiments with fluorescently labeled protein tau were conducted. b,c. FCS analyses of particle number (B) and particle brightness (C) of SUV488. The stable particle number and brightness at high tau concentrations indicates that the increase in diffusion time is more likely due to protein binding (and possibly subsequent aggregation) than vesicle fusion or crosslinking. d,e. Exemplary autocorrelation curves of POPS-SUV (d) and POPC-SUV (e) in the presence and absence of unlabeled protein tau. Only POPS-SUV demonstrate a marked prolongation of the autocorrelation signal. * = $p<0.05$; n=3.



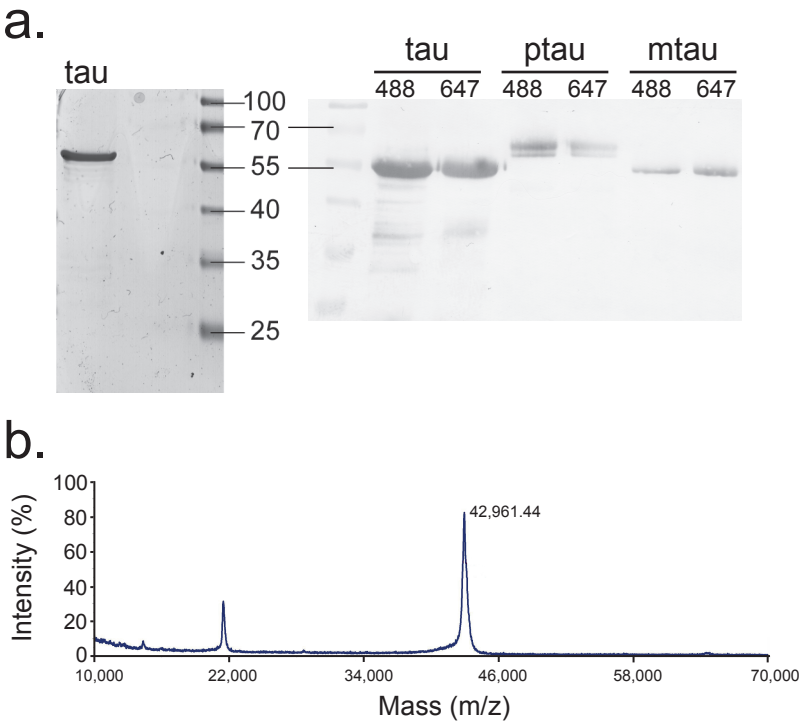
Supplement figure S2
A. 2D FIDA histograms depicting asyn monomer binding to DPPC-SUV. After addition of SDS 0.2%, DPPC-SUV are completely dissolved. No asyn oligomers can be observed along the ordinate. B. Al3+ induced asyn and tau oligomers bind to POPC-SUV, and are stable after dissolution of vesicles by SDS 0.2%.

supplement figure S 3

Cross-correlation analysis of oligomer vesicle-binding. A. While both tau and asyn oligomers induced by trivalent metal ions show binding to POPC-SUV (left panel) and DPPC-SUV (right panel), only asyn monomers bind to DPPC-SUV. Exemplary cross-correlation curves (single experiment, average of 4 independent samples) are provided below. B. Phosphorylated protein tau oligomers demonstrate increased binding to POPC-SUV and DPPC-SUV. Again, cross-correlation curves from a single experiment are provided below the panels. ‡= p < 0.001



Supplement figure S4
A. As described previously, tau phosphorylation with GSK-3β enhances tau oligomer formation induced by trivalent metal ions. B. While oligomer formation is increased after tau phosphorylation, oligomer sizes as determined by 1D-FIDA analysis do not differ significantly. * $p < 0.05$; $n = 3$.



Supplement figure S5

Protein tau quality controls. a. Coomassie gel (left) of unlabeled protein tau. The right panel demonstrates the typical band shift of fluorescently labeled protein tau phosphorylated by GSK-3β (ptau), whereas mock-phosphorylated protein (mtau) runs at the same height as the unphosphorylated protein (tau). b. Mass spectrometry demonstrating that the recombinant protein tau is free of contaminants.