Supplementary Material

Phosphorylation of the amyloid-beta peptide inhibits zinc-dependent aggregation, prevents Na,K-ATPase inhibition, and reduces cerebral plaque deposition

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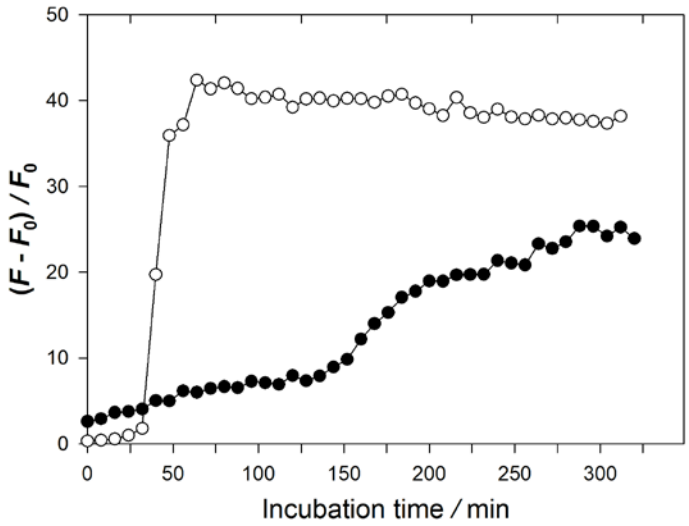
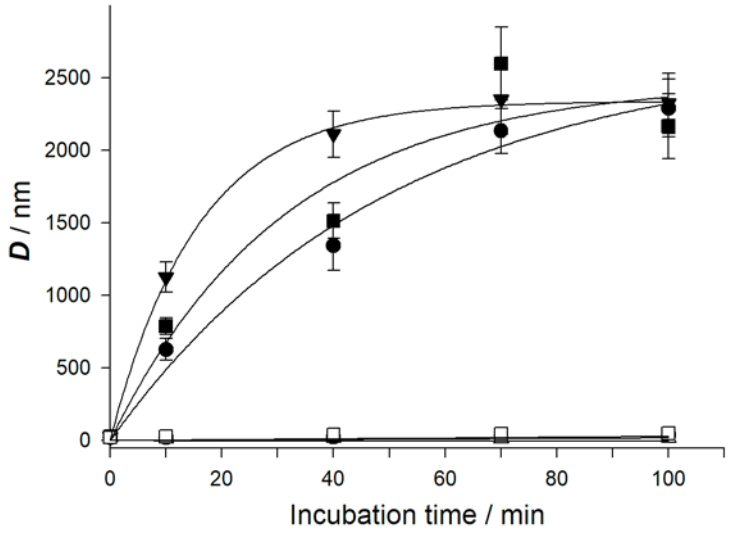
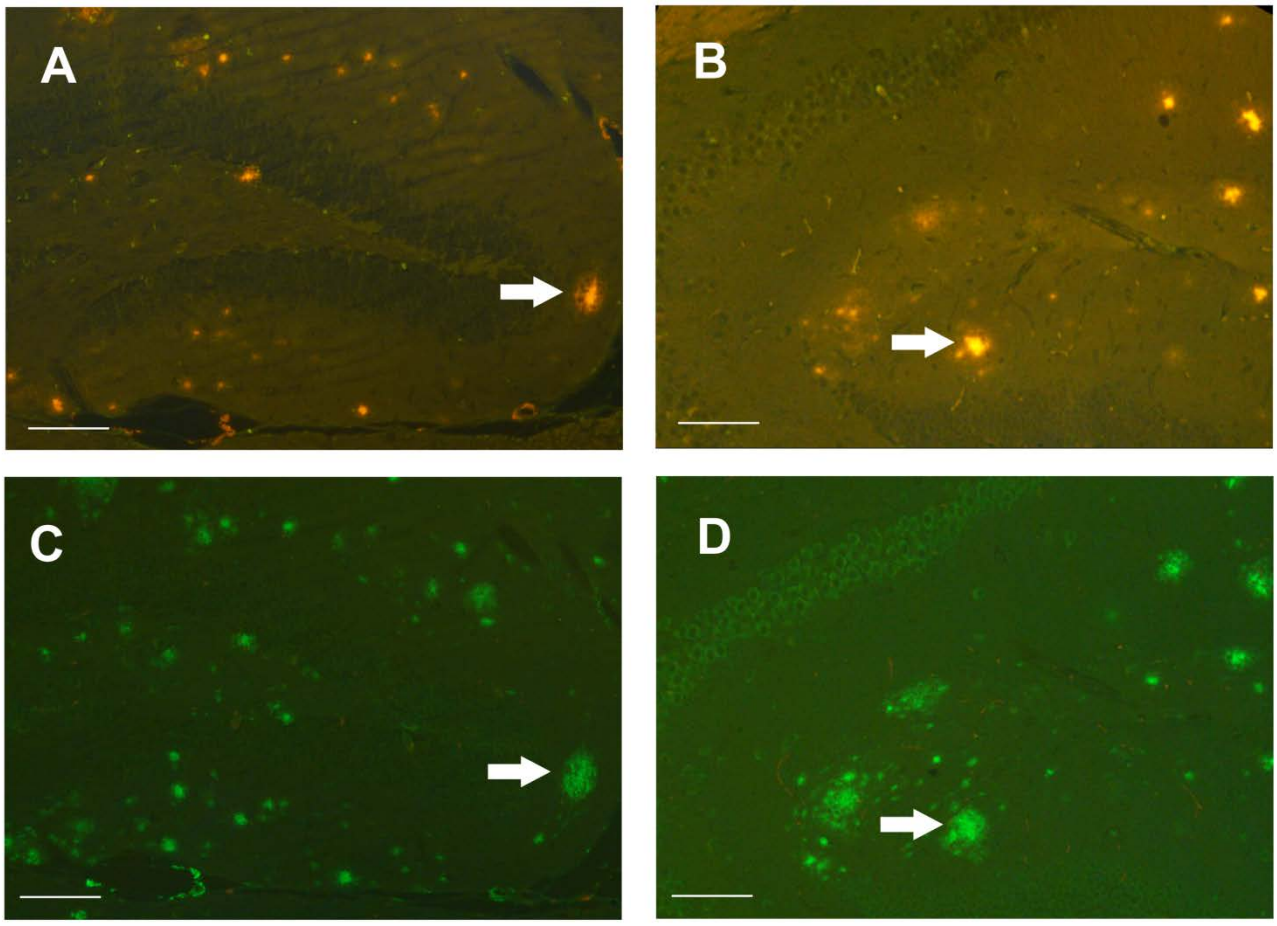
Supplementary Figures



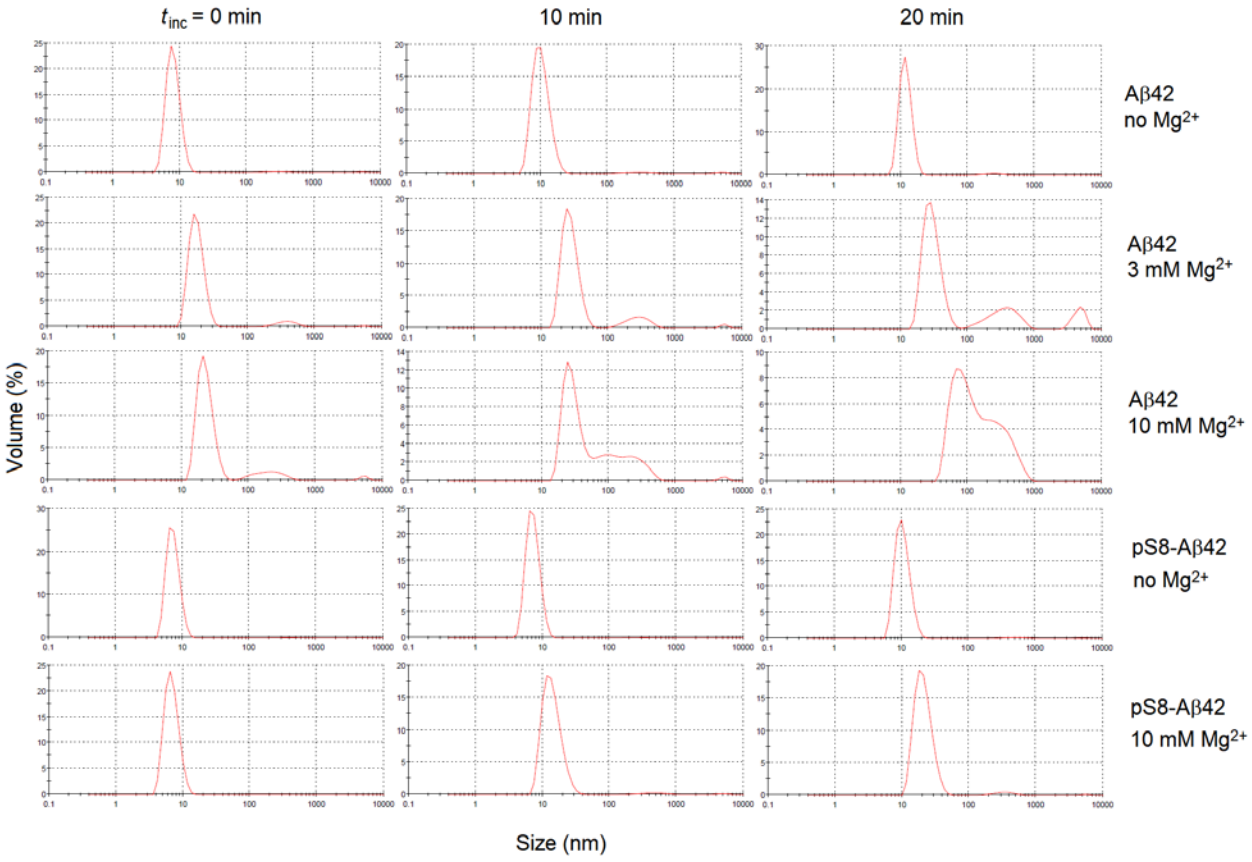
Supplementary Figure 1. Thioflavin T fluorescence $(F-F_0)/F_0$ in solutions of $A\beta_{42}$ peptide (black symbols) and pS8-$A\beta_{42}$ peptide (white symbols) was monitored over 300 min. Fluorescence measurements were carried out on the Infinite M200 PRO microplate reader (TECAN, Switzerland) using Corning 96-well microplates. The excitation and emission wavelengths were set at 450 and 482 nm, respectively. To test the self-aggregation of Aβ peptide isoforms, 100-µL aliquots of Aβ solutions (peptide concentration – 30 µM) were mixed in wells with 20 µL of the thioflavin T (ThT) solution in buffer H (ThT concentration – 150 µM), followed by the incubation at 37°C with constant agitation. The fluorescence measurements were started immediately after preparation of Aβ/ThT mixtures. Values of fluorescence in wells containing buffer alone were subtracted from those in wells containing ThT. The relative changes of ThT fluorescence intensity were calculated as (F - F0) / F0, where F and F0 are fluorescence intensities of ThT in the presence and absence of Aβ peptides, respectively. The mean values of triplicate measurements are shown.



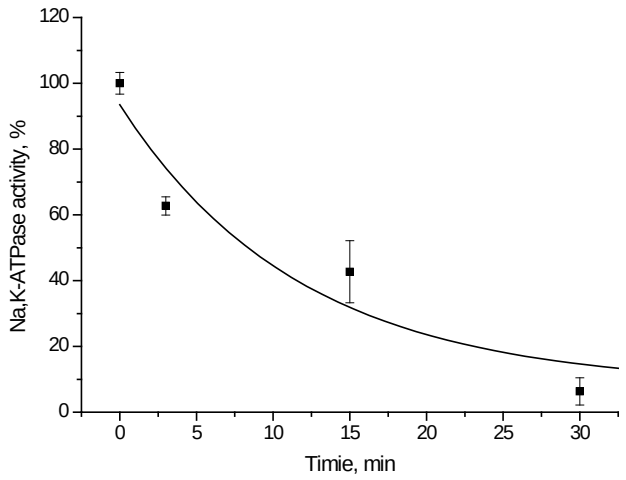
Supplementary Figure 2. Characteristic diameter (*D*) of zinc-induced aggregates of $A\beta_{42}$ and pS8-$A\beta_{42}$ peptides was monitored with DLS over 100 min. black symbols – $A\beta_{42}$, white symbols – pS8-$A\beta_{42}$. Circles, squares, and triangles – zinc/peptide molar ratios of 1, 2, and 3, respectively. Peptide concentration – 25 μM. Buffer – 10 mM HEPES (pH 7.4), 50 mM NaCl. Data are mean values for three independent experiments ± SD. Size of some symbols is larger than the error bars.



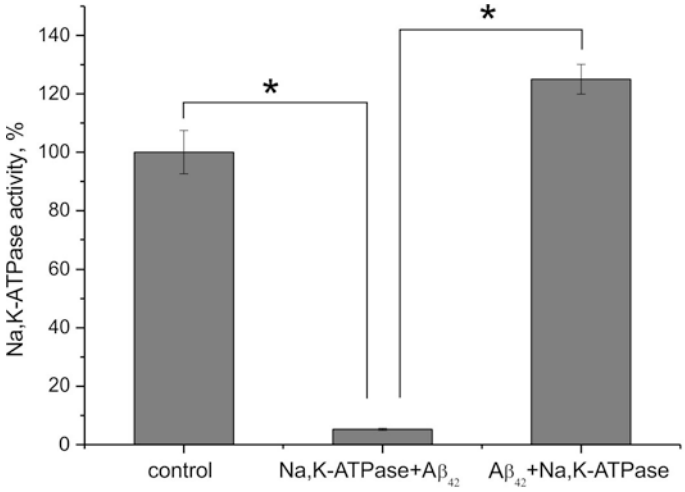
Supplementary Figure 3. Representative fluorescent micrographs of brain sections through the hippocampus for 8-month-old B6C3-Tg(APPswe,PSEN1dE9)85Dbo/j transgenic mice intravenously injected with sterile physiological saline (**A, C**) or synthetic pS8-$A\beta_{42}$ peptide (**B, D**). Counterstaining of adjacent sections of the brain in the dentate gyrus of the hippocampus by Congo Red dye (**A, B**), and immunohistochemical staining by specific antibodies to Aβ (**C, D**). Selected amyloid inclusions are indicated by white arrows. Scale bars: (**A, B, C, D**) 100 μm.



Supplementary Figure 4. The graphic output of the Zetasizer Nano ZS apparatus: Representative size distributions by volume (fraction of total particle volume, %, occupied by particles of a given diameter) for $A\beta_{42}$ and pS8-$A\beta_{42}$ isoforms in the absence and the presence of magnesium ions at various incubation times. Peptide concentration – 25 μM. Buffer – 10 mM HEPES (pH 7.4), 150 mM NaCl. DLS measurements were started immediately after the addition of magnesium ions. Peptide solutions were incubated at 25°C under quiescent conditions.



Supplementary Figure 5. Effect of $A\beta_{42}$ on hydrolytic activity of Na,K-ATPase in solution. Hydrolytic activity of Na,K-ATPase after 0, 3, 15, 30 min incubation with 30 μM $A\beta_{42}$. Enzyme activity without $A\beta_{42}$ is accepted as 100%. Data are mean values for three independent experiments ± SD. Curve was fitted by one phase exponential decay equation using Origin program.



Supplementary Figure 6. Effect of $A\beta_{42}$ on hydrolytic activity of Na,K-ATPase in solution. The hydrolytic activity of Na^+,K^+-ATPase was measured after 30 min incubation with 40 μM of $A\beta_{42}$ (Na,K-ATPase + $A\beta_{42}$). Alternatively, 30 μM $A\beta_{42}$ was incubated in a solution for 30 min, after which Na^+,K^+-ATPase was added and its activity was measured immediately ($A\beta_{42}$+Na,K-ATPase). Enzyme activity without $A\beta_{42}$ is accepted as 100%. Data are mean values for three independent experiments ± SD. Statistical analysis was performed using one-way ANOVA ($F=414.4$, degree of freedom 2, $P<0.00001$) with post-hoc testing (using paired samples Student's t-test with Bonferroni correction); after a Bonferroni correction, a P value <0.016 was considered as statistically significant; * $P< 0.001$.