

Additional file 8:

SDS-PAGE and Western Blot

A mix of synthetic A β -peptides (A β ₁₋₃₇, A β ₁₋₃₈, A β ₁₋₃₉, A β ₁₋₄₀ and A β ₁₋₄₂) (AnaSpec) as well as cell culture supernatant of SH-SY5Y APP695 cells containing secreted A β -peptides was separated on 10% T/5% C polyacrylamide gels by urea bicine/tris SDS-PAGE (Klafki, 1996). The separated peptides were electrophoretically transferred onto PVDF-membranes (Biorad) at 0.75 mA/cm² under semi-dry conditions for 30 min. In order to improve antibody binding, the PVDF-membranes were then boiled in pre-warmed PBS for 3 min in a microwave. Afterwards, the membranes were blocked with 2% ECL prime blocking reagent (GE Healthcare) in PBS-T for 1h at room temperature. Immunostaining was performed with the anti-A β antibody 1E8 (Nanotools) overnight at 4°C. The next day, the membranes were washed three times in PBS-T before they were incubated with a biotinylated anti-mouse IgG (Linaris) for 1h at room temperature. Then, membranes were washed again in PBS-T before incubation with a streptavidin horseradish peroxidase complex in PBS-T (GE Healthcare). The membranes were developed with ECL-plus chemiluminescence (GE Healthcare) according to the manufacturer and the bands were visualized using a Fusion SL Imager (Vilber).

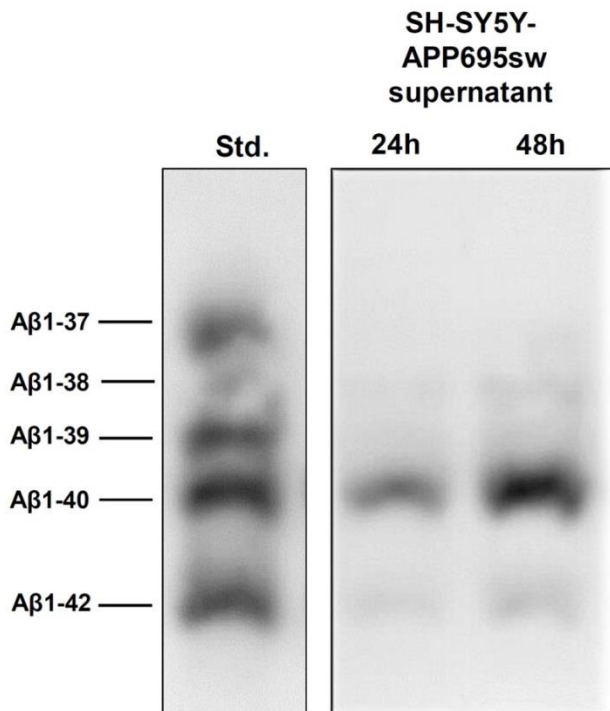


Figure S2: Medium from APP695sw-transfected SH-SY5Y cells contained mainly A β ₁₋₄₀ and A β ₁₋₄₂ after 48h.