Supporting Information

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SI Text

Materials and Methods. ***Conditions of amyloid fibril formation in vitro.*** Human insulin (Sigma): 1 mg/mL, H_2O/HCl, pH 2.0, 7 d, 60 °C. Human glucagon (Sigma): 1 mg/mL, 50 mM sodium phosphate, pH 3.0, 7 d, 4 °C. Murine serum amyloid A protein (mSAA) from in house recombinant expression (1): 10 mg/mL, 50 mM sodium phosphate, pH 2.0, 1 d, 65 °C. Human transthyretin (gift from J. Kelly): 1 mg/mL, 50 mM sodium phosphate, pH 2.0, 4 d, 37 °C. Aβ(1–40) from in house recombinant expression (2): 1 mg/mL seeded with 1% synthetic Aβ(1–40) (Bachem) 50 mM sodium borate pH 9.0, 2 d, 20 °C; Aβ(1-42) (Bachem): 0.2 mg/mL, 10 mM sodium borate, pH 9.0, 2 d, 20 °C. Fibrils from horse apomyoglobin (Sigma) and myoglobin G-helix (Jerini, custom peptide synthesis) were prepared as described (3, 4). Concentrations refer to gravimetrical determinations. Presence of fibrils was confirmed by electron microscopy.

Thioflavin-T (ThT) aggregation kinetics measurements. Unless indicated otherwise, samples contained 50 μM Aβ(1–40) in 100 μl 50 mM Hepes buffer, pH 7.4, 20 μM ThT, 10 mM sodium azide with or without 0.1 μg seeds. Instrumental settings were used as described (5). Fibrillation curves consist typically of two phases, termed lag phase and growth phase. The length of the lag phase reports on the nucleation efficiency; i.e., a long lag phase means slow nucleation, a fast lag phase means fast nucleation. The efficiency of the growth phase is typically quantified by fitting an exponential function to the growth part of the curve and by extraction of the growth rate constant. Details on measurement of the lag time and growth rate constant can be found elsewhere (6).

Preparation of ^{488}A. 2 mg PromoFluor 488 (PromoKine, Heidelberg) were dissolved in 1.6 mL dimethylsulfoxide and added drop-wise to 7.13 mL of a solution of 0.1 M sodium bicarbonate (pH 7.0) containing 4.64 mg Aβ(1–40). The mixture was stirred over night and purified by gel filtration (Superdex75 HiLoad, GE Healthcare), run with 50 mM sodium phosphate buffer (pH 7.0), 0.1 M NaCl. ^{488}Aβ was dialyzed over night (4° C) against water using a Spectra/Por DispoDialyzer (MWCO 500 Da, Spectrum Laboratories) and lyophilized. Mass spectrometry shows a single species (4800.4 Da). Theoretic mass of Aβ(1–40) labeled with one PromoFluor 488 molecule: 4801.3 Da.

Amyloid plaque formation in cell culture. Primary murine macrophages or microglia were obtained from Dr. A. Kleyman (FLI, Jena, Germany) and M. Strassburger (IfN, Magdeburg, Germany). Primary cells were plated out on glass slides placed within 6- or 24-well plates (TPP) and cultured in DMEM medium (Invitrogen) supplemented with 10% foetal bovine serum (FBS, Invitrogen) and 20% L929 cell-conditioned medium as a source of mononuclear phagocyte colony-stimulating factor (M-CSF).

Cell lines were plated out at a density of 10^5 cells/mL in 96-well plates (Cellstar, Greiner-Bio One) or on glass slides placed within 6- or 24-well plates. Incubation was carried out at 37 °C with 5% (v/v) CO_2. Culture media and supplements were exchanged every other day. RPMI medium (PAA) was used for THP-1 cells and DMEM medium (PAA) for all other cell lines. All media were supplemented with 10% (v/v) FBS (PAA), 100 units/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B (PAA). THP-1 cells were differentiated into macrophages with 100 ng/mL phorbol 12-myristate 13-acetate (PMA) (Calbiochem). Before plaque formation was induced, the adherent primary cells were incubated for 8 days. Then they were rinsed carefully with PMA-free and M-CSF-free medium. All cell lines were incubated for 1 day before plaque formation was induced.

Plaque formation was induced by supplementing the culture medium with freshly dissolved Aβ(1–40) peptide (final concentration in the well: 60 μg/mL). Immediately before Aβ was added to the medium, lyophilized, recombinant Aβ(1–40) peptide was dissolved at 10 mg/mL concentration in double-distilled water, sonicated for 2 min, followed by centrifugation for 15 min $(10,500 \times g)$. Freshly dissolved Aβ was replenished each time the culture medium was replaced. Previous experiments have shown that the properties of freshly dissolved, recombinant Aβ(1–40) peptide correspond well to Aβ peptide samples that were subjected to so-called disaggregation protocols (6) and that are frequently said to contain "monomeric" Aβ. However, the true oligomerisation state after addition to the cell culture medium is not known. Therefore, we only refer to freshly dissolved Aβ peptide and not to monomeric Aβ.

In some experiments the medium was additionally supplemented with seeds. Whenever seeds were added, this was done only on the first day that freshly dissolved Aβ was added. Seeds were not replenished, when the culture medium was replaced. Seeds normally represent recombinant Aβ(1–40) fibrils that were formed in vitro (see previous section). These fibrils were dialysed over night (4 °C) against phosphate buffered saline (PBS), using a Spectra/Por DispoDialyzer (500 Da MWCO; Spectrum Laboratories). Aliquots from the dialysed solution were flash frozen and thawed immediately before usage. In the cross-seeding experiment (Fig. S4) also non-Aβ fibril preparations were used as seeds.

Lactate dehydrogenase (LDH) assay. THP-1 cells were incubated up to 5 days with Aβ(1–40) (final Aβ concentration 60 μg/mL). Cytotoxicity was measured using the LDH-Cytotoxicity Assay Kit II (BioVision).

Plaque purification. Plaque formation was induced in a THP-1 cell culture by addition of 60 μg/mL freshly dissolved Aβ(1–40) peptide and 100 ng/mL seeds, corresponding to 0.2% of the total amount of Aβ peptide added on day zero. After 13 days, plaques were scraped off in pure water, frozen in liquid nitrogen and stored at −20 °C until further use.

Congo red green birefringence. CR green birefringence was carried out as described previously (1). In brief, cells grown on coverslips were washed thoroughly with phosphate-buffered saline, fixed in ice-cold methanol for 10 min and stained for 45 min with filtered CR solution [80% ethanol, 3% NaCl, 0.6% CR]. Cells were then washed three times with water and incubated for 2 min with Mayeŕs hämalaun solution (Roth), washed once with 70% ethanol and trice with water. The cells were then dehydrated with 90% ethanol, 100% ethanol and finally with xylol before they were mounted with Roti-Histokitt (Roth) and examined with a Zeiss Axiovert 200 Apotome.

Filter retention assay. Amyloid quantification was carried out as described (7). In brief, cells in 96-well plates were washed thoroughly with phosphate-buffered saline, lysed in 50 μl lysis buffer [50 mM Tris HCl (pH 8.8), 100 mM NaCl, 5 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid, 0.5% (w/v) Nonidet P-40] containing a protease inhibitor mix (Roche Diagnostics) for 30 min on a shaking device (approximately 500 rpm) at 4 °C. After adding 50 μl of water and 100 μl of 4% sodium dodeclysulfate in

100 mM dithiothreitol, the solution was boiled for 2 × 7 min at 98 °C and filtered through a 0.2 μm cellulose acetate membrane (Schleicher and Schuell) with a 96-well vacuum dot-blot apparatus (Schleicher and Schuell), followed by three washing steps with 0.1% SDS. The membrane was blocked for 1 h in 20 mM Tris-HCl, pH 7.4, 137 mM NaCl, 0.1% Tween 20 containing 2% BSA. Aggregates were detected by incubation with anti-Aβ (1–16) primary antibody (Chemicon; 1:200 in blocking buffer) for 1 h, followed by incubation with AP conjugated anti-mouse secondary antibody (1:15,000 in blocking buffer; Sigma) for another 1 h. Blots were developed with LumiPhos reagent according to the manufacturer's instructions (Pierce), and the signals were scanned and quantitatively analyzed with TotalLab100 densitometric software (Nonlinear Dynamics).

Alcian Blue staining. The staining with Alcian Blue was carried out as described previously (7).

Time-lapse video microscopy. THP-1 cells were incubated for 2 days in a 24-well plate, differentiated 1 day before placing them in a custom-made chamber (Incubator S-M, Pecon) that is mounted within an Axiovert 200 M (Zeiss) microscope. The medium was supplemented with a final concentration of 60 μg/mL Aβ(1–40), consisting of a 10:1 mixture of freshly dissolved Aβ(1–40) and freshly dissolved ^{488}Aβ. Each well was supplemented 100 ng/mL seeds, corresponding to 0.2% of the total amount of Aβ peptide added on day zero. Incubation was carried out at 37 °C in 5% CO_2. Fluorescence and phase contrast images were recorded at 10 min intervals with a CCD-camera (Axiocam MRm; Zeiss). Data analysis was performed with Zeiss software (AxioVs40 V4.5).

Confocal and confocal-like fluorescence microscopy. Primary antibodies: Aβ(1–16) (1:200 in blocking buffer, Chemicon), anti-EEA1 (1:500, BD Bioscience), anti-Lamp2 (1:300, H4B4 Developmental Studies hybridoma Bank at the University of Iowa), anti-giantin (1:1000, Alexis Biochemicals), or anti-calnexin (1:300, Stressgen Biotechnologies). Calnexin staining: THP-1 cells were incubated with 60 μg/mL Aβ(1–40) for 2 h before fixation and antibody staining (Aβ(1–16) and anti-calnexin). EEA1, Lamp2 and giantin staining: THP-1 cells were incubated at 37 °C with 60 μg/mL of ^{488}Aβ for 2 h before fixation and antibody staining (anti-EEA1, anti-Lamp2 and anti-giantin). Transferrin staining: THP-1 cells were incubated at 37 °C with 5 μg/mL ^{555}Tf (Molecular Probes) and 60 μg/mL of ^{488}Aβ for 5 min before fixation. Dextran and Lyso Tracker staining: THP-1 cells were incubated at 37 °C with 1 mg/mL rhodamine B isothiocyanate-Dextran (Sigma) and 120 μg/mL of ^{488}Aβ or with 60 nM Lyso Tracker Red DND-99 (Invitrogen) and 120 μg/mL ^{488}Aβ. Fixation: after washing with PBS cells were fixed in 4% PFA, washed, stained with Hoechst 33342 (nucleus staining) and mounted on glass slides. In case of antibody stainings cells were additionally washed thoroughly with PBS and blocked for 30 min in blocking buffer (2% BSA in PBS). Non-specifically bound primary antibodies were washed off thoroughly with PBS before the addition of a secondary antibody (goat anti mouse or anti rabbit coupled with Alexa Fluor 488 or Alexa Fluor 555; 1:200 in blocking buffer) for 30 min, followed by DNA staining with Hoechst 33342 and washed with PBS. Images were recorded with a Zeiss Axiovert 200 Apotome (confocal-like microscopy) using standard fluorescence filters for DAPI (Hoechst 33,342), GFP and Rhodamine or with a Zeiss LSM510 (confocal microscopy). Exposure times and gain settings were carefully chosen to minimize cross talk between channels.

Scanning electron microscopy. Plaque formation was induced in a THP-1 cell culture by addition of 60 μg/mL freshly dissolved Aβ(1–40) peptide and 100 ng/mL seeds, corresponding to 0.2% of the total amount of Aβ peptide added on day zero. After incubation for 6 days on glass slides, cells were immunolabelled and prepared for scanning electron microscopy as described (8). Cells were labelled with an Aβ(1–16) antibody diluted 1:25 and a second goat anti-mouse antibody coupled with 20 nm gold, 1:50 (British Biocell International). The cells were examined in a field emission scanning electron microscope (FE-SEM, LEO 1530 Gemini) at 5 kV acceleration voltage and a working distance of 7 mm using a secondary electron detector and a scintillation type backscatter electron detector. For immunogold detection secondary and backscatter detector images were mixed with 20:80 ratio.

Freeze-fracture replica immunogold labeling. Plaque formation was induced in a J-774A.1 cell culture by addition of 60 μg/mL freshly dissolved Aβ(1–40) peptide and 100 ng/mL seeds, corresponding to 0.2% of the total amount of Aβ peptide added on day zero. Cells were incubated up to 6 days in 75 cm^3 culture flasks, scraped off and concentrated by centrifugation. Aliquots were enclosed between two 0.1 mm copper profiles and rapidly frozen by plunging them into liquid propane cooled by liquid nitrogen. The samples were fractured and replicated in a BAF400T (BAL-TEC) freeze-fracture unit at −150 °C using a double-replica stage. The replica immunogold labeling was performed as described previously (9), using an Aβ(1–16) antibody diluted 1:25 and a 1:50 diluted second goat anti-mouse antibody coupled with 10 nm gold (British Biocell International). After immunolabeling, the replicas were picked onto EM grids for viewing in an EM902A transmission electron microscope (Zeiss).

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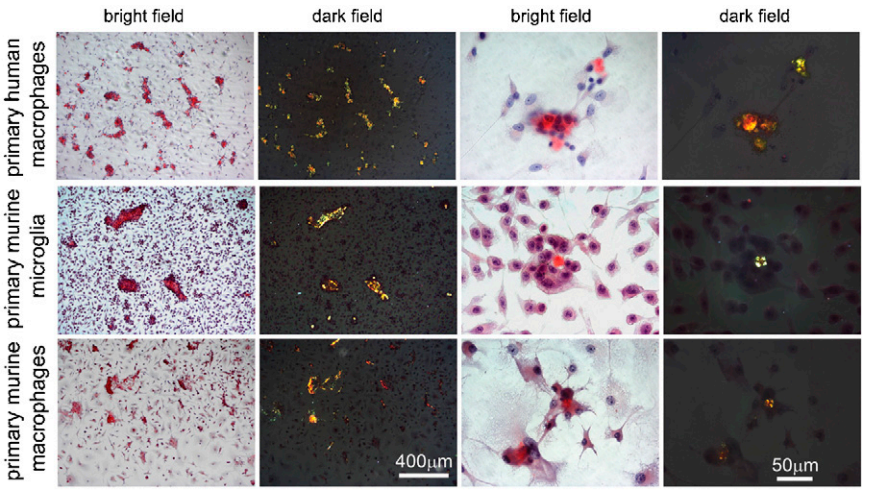

Fig. S1. Different primary mononuclear phagocytes promote Aβ amyloid plaque formation. Primary human macrophages from peripheral blood, primary murine macrophages from bone marrow and primary murine microglia were incubated for 8–10 days with in the presence of freshly dissolved 60 μg/mL Aβ(1–40) but without addition of seeds. Images show polarizing microscopy pictures (bright field and dark field) after staining with CR and Mayer's hämalaun. Left image pair: overview; right image pair: close up.

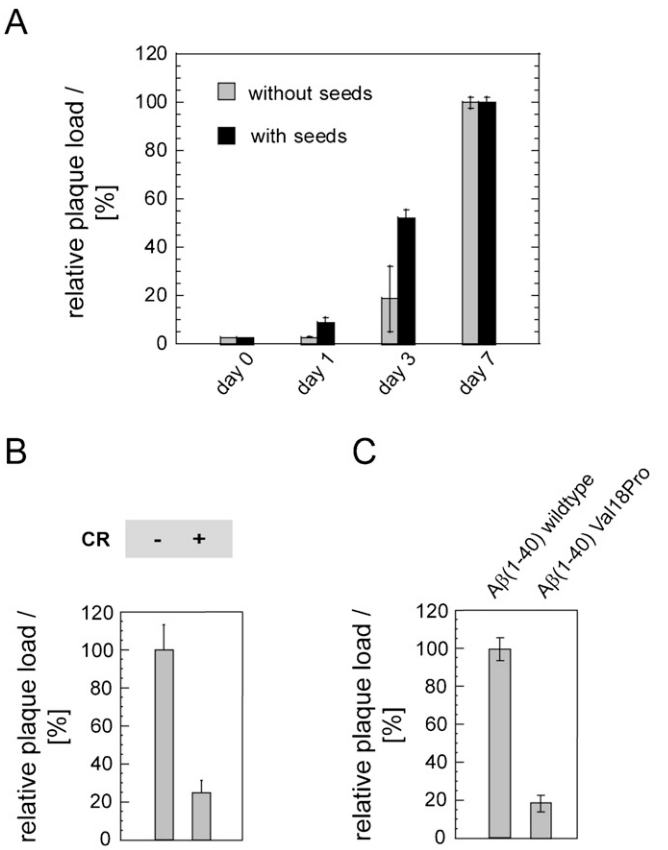

Fig. S2. Effect of seeding, Congo red and Aβ-mutation on the plaque yield. (*A*) Amyloid load obtained of a THP-1 cell culture after different time points in the presence of freshly dissolved 60 μg/mL Aβ(1–40) with or without seeds. At time point 0 min, the medium of the seeded wells was additionally supplemented with 100 ng/mL seeds, corresponding to 0.2% of the total amount of Aβ peptide added on day zero ($n = 6$). (*B*) Amyloid load of a THP-1 cell culture after 2 d incubation in the presence of 10 μM CR and 60 μg/mL freshly dissolved Aβ(1–40) ($n = 8$). (*C*) Amyloid load of a THP-1 cell culture after 4 d incubation in the presence of 60 μg/mL freshly dissolved wildtype Aβ(1–40) or Aβ(1–40)Val18Pro ($n = 8$). The culture media in (*B*) and (*C*) were additionally supplemented on day zero with 100 ng/mL seeds, corresponding to 0.2% of the total amount of Aβ peptide added on day zero. Panels show densitometric quantifications: 0%: background; 100%: amyloid load with wildtype Aβ(1–40) and without CR.

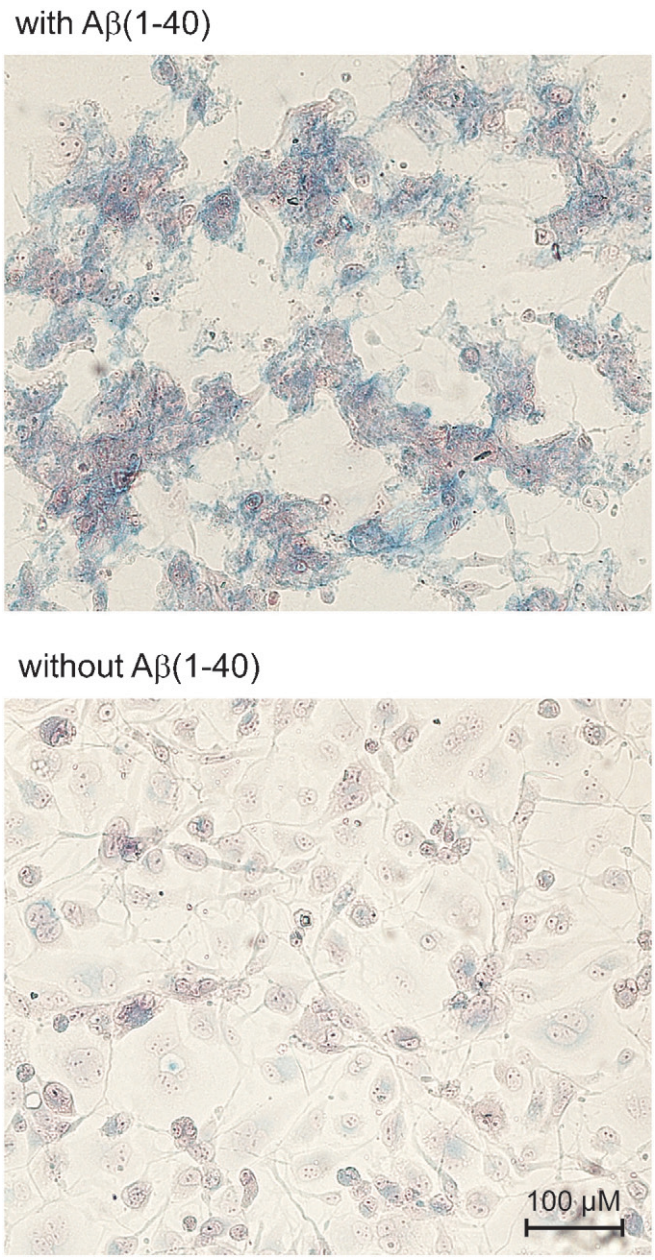

Fig. S3. Glycosaminoglycane staining with Alcian blue. THP-1 cells incubated with or without 60 μg/mL Aβ(1–40) for 4 days. On day zero, the medium of the Aβ containing culture was additionally supplemented with 100 ng/mL seeds, corresponding to 0.2% of the total amount of Aβ(1–40) peptide added on day zero. Aβ-treated cells show Alcian blue positive staining of plaques. Slides were counterstained with 0.1% (wt/vol) nuclear fast red solution (Sigma).

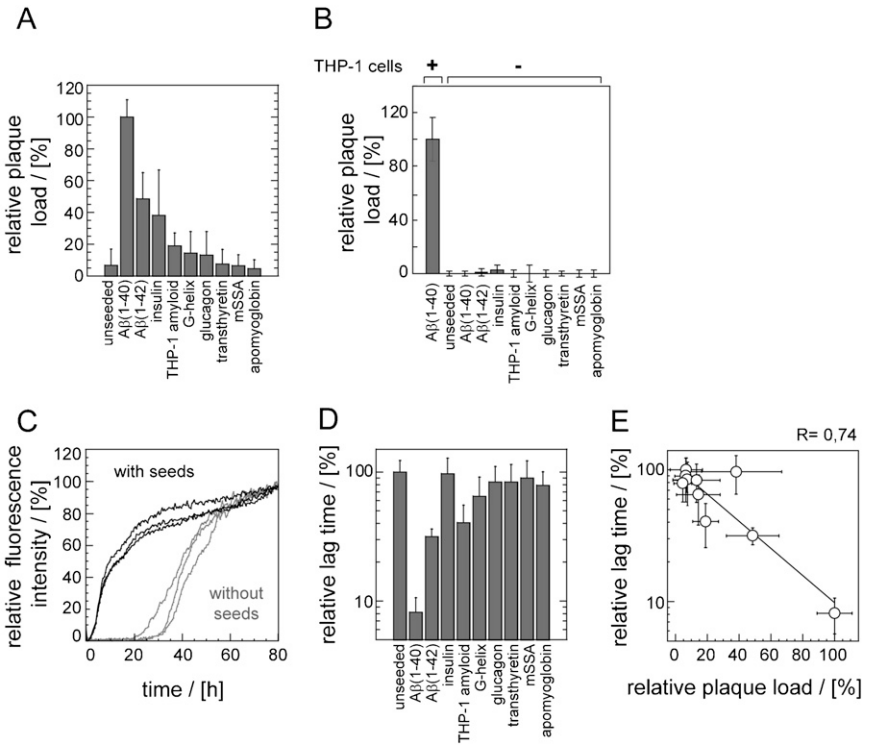

Fig. S4. Template-dependence of plaque formation. (*A*) Plaque yield in a THP-1 cell culture obtained with different seeds ($n = 28$). The culture medium was supplemented with 60 μg/mL Aβ(1–40) and seeds as indicated. Seeds were only added on day zero and at 250 ng/mL concentration, corresponding to 0.4% of the amount of Aβ peptide added on day zero. Seeds represent samples of amyloid fibrils that were formed in vitro or samples of amyloid plaques purified from a previous THP-1 cell culture (THP-1 amyloid). (*B*) Same as in *A* but without cells ($n = 24$). (*C*) Aβ(1–40) in vitro fibrillation assay monitored by thioflavin-T fluorescence. Kinetics traces show three replicates of a solution of 50 μM freshly dissolved Aβ(1–40) peptide with or without 0.1 μg Aβ(1–40) amyloid fibrils that were preformed in vitro. This amount of seeds corresponds to 0.5% of the freshly dissolved Aβ peptide. (*D*) Relative lag time from Aβ(1–40) in vitro fibrillation assays with or without different seeds (0.1 μg seeds per well; $n = 24$). The lag time of an unseeded Aβ(1–40) sample was set to 100%. (*E*) Correlation of the data sets shown in *A* and *C*.

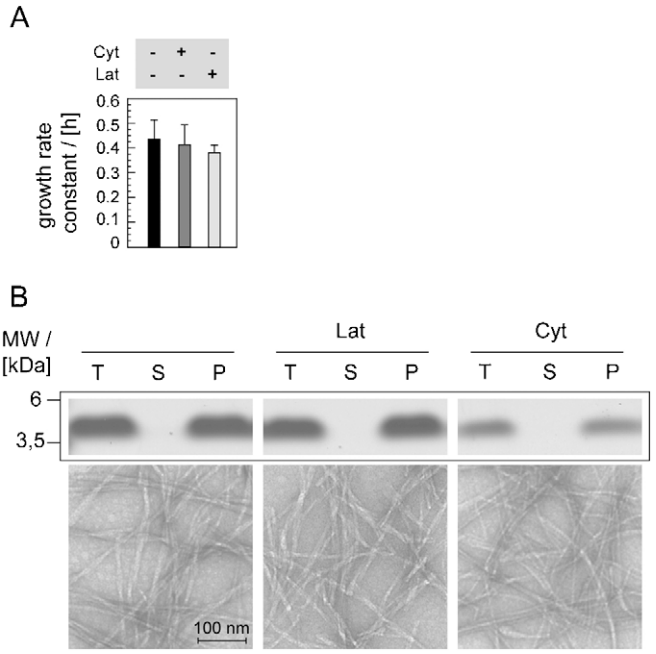

Fig. S5. Inhibition of Aβ(1–40) aggregation by cytochalasin B (Cyt) and latrunculin B (Lat). (*A*) Effect of Cyt or Lat on the kinetics of Aβ(1–40) fibril growth, determined by the growth rate constant ($n = 8$). The growth rate is obtained by fitting the growth part of the in vitro fibrillation kinetics curve (6). Within the error of the experiment, Cyt or Lat do not affect the growth rate constant. Control reactions confirm that 6 μM Cyt or 2.5 μM Lat block uptake of freshly dissolved, soluble or fibrillar ^{488}Aβ or fluorescent beads into THP-1 cells. (*B*) Effect of Cyt or Lat on the extent of Aβ(1–40) fibril formation in terms of formation of insoluble Aβ(1–40). Image shows the Aβ band from a Coomassie-stained sodium dodecylsulfate polyacrylamide gel. Samples represent total (T) sample of freshly dissolved 50 μM Aβ(1–40) incubated for 17 days in 50 mM Hepes buffer, pH 7.4, (37 °C), as well as the supernatant (S) and redissolved pellet (P) after ultracentrifugation at 513,000 g (4 °C, 120 min). Electron microscopy confirms the presence of almost exclusively fibrillar aggregates with or without Cyt or Lat.

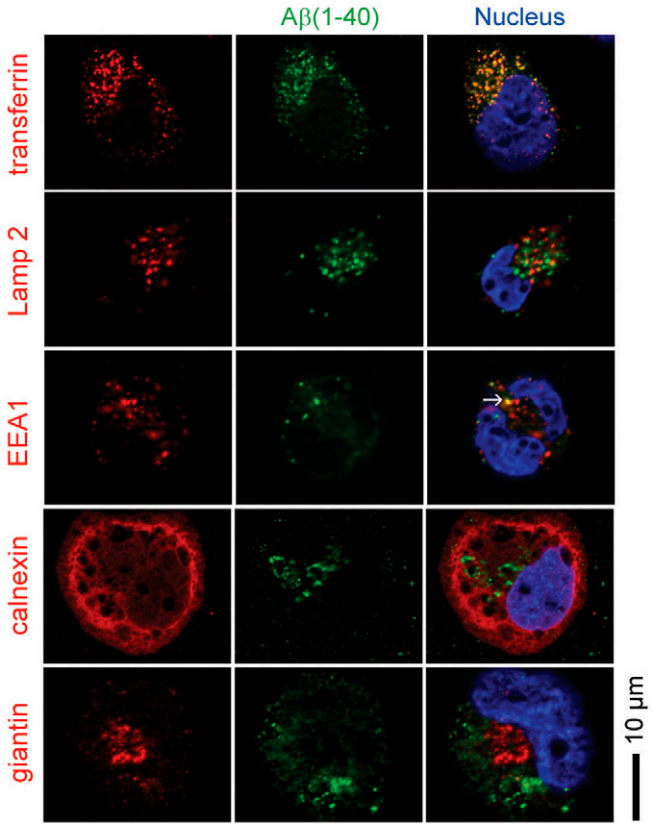

Fig. S6. Subcellular localisation of Aβ within the endocytic pathway. THP-1 cells were incubated with ^{488}Aβ for indicated time periods and stained for immunofluorescence microscopy. Early endosomes were visualised by co-incubation with ^{555}TF or staining with EEA1 antibodies. Aβ shows weak colocalization with early endosomal antigen 1 (EEA1) or the lysosomal marker Lamp2. Aβ does not colocalize with markers for either Golgi apparatus (giantin) or endoplasmic reticulum (calnexin). (*Blue*): nuclear staining.

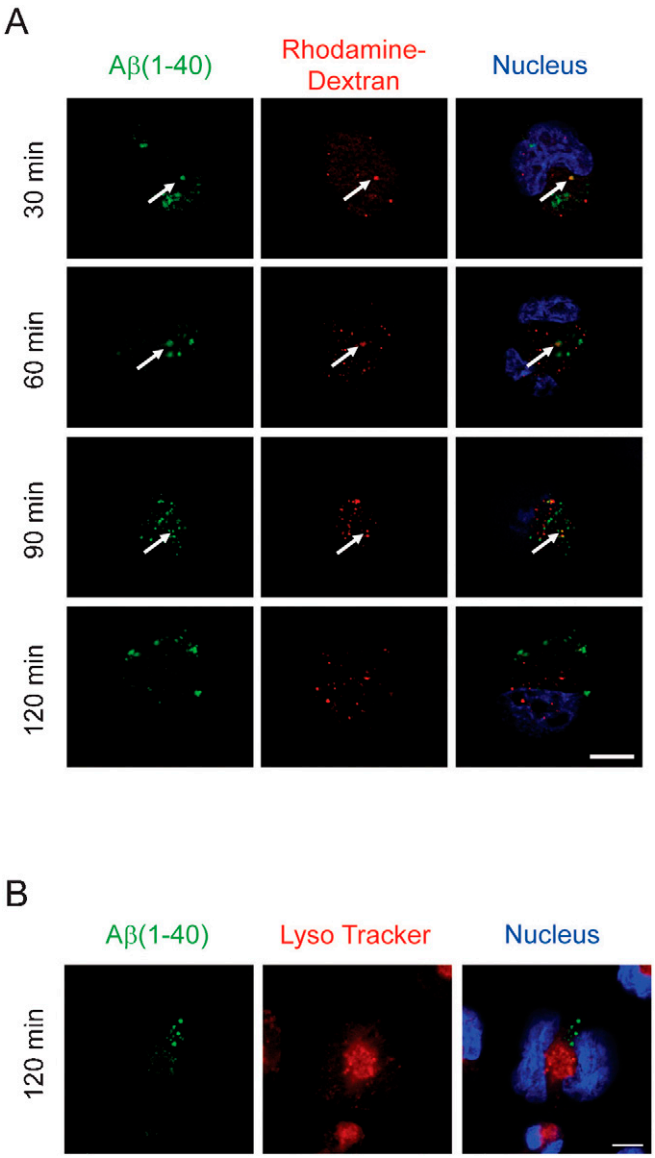

Fig. S7. Aβ-containing vesicles do not colocalize with lysosomal markers. (*A*) THP-1 cells were incubated with ^{488}Aβ and rhodamine-dextran for indicated time points, fixed and analyzed by confocal-like microscopy. Single confocal-like sections shown. The images show some colocalization of rhodamine-dextran and Aβ (highlighted by arrows). After 2 h only very little, if any, co-colocalisation is seen. (*B*) THP-1 cells were incubated for 2 h with ^{488}Aβ and Lyso Tracker, fixed and analyzed by confocal microscopy. Single confocal sections shown. The images show no colocalization of Lyso Tracker and Aβ. (Scale bar: 10 μm.) *Blue*): nuclear staining.

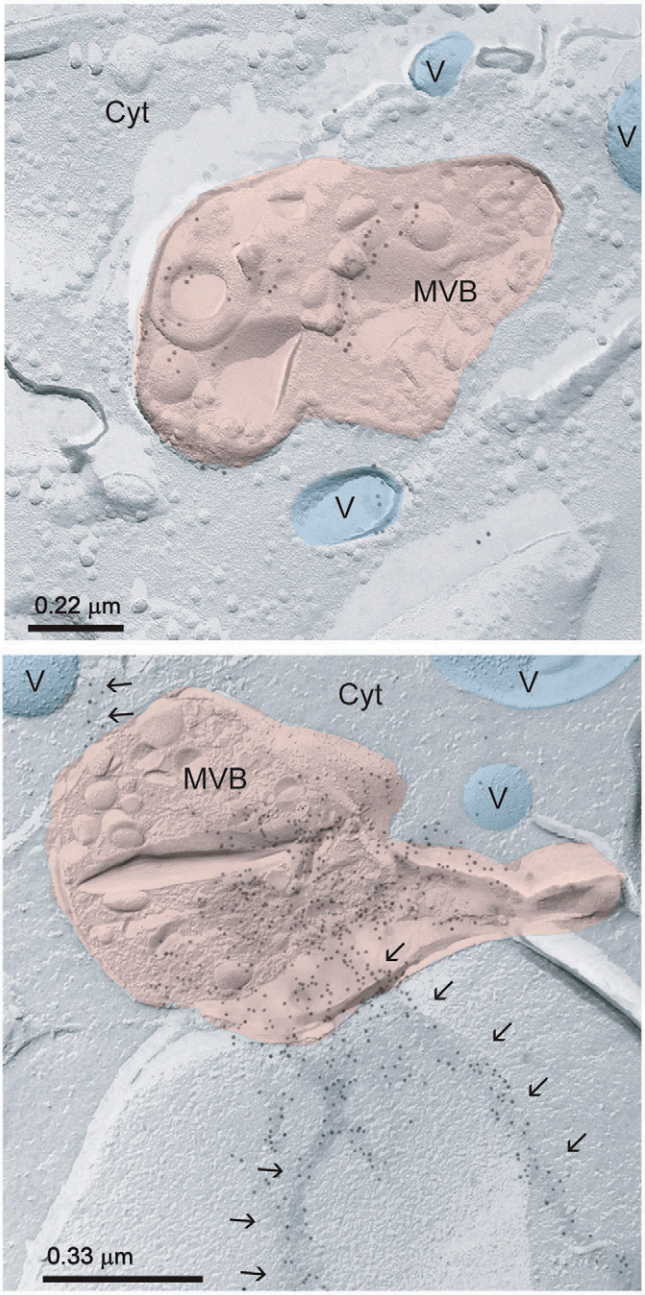

Fig. S8. Aβ-dependent perturbation of the MVBs in freeze-fractured cells. TEM images of MVBs enclosing Aβ (*Upper*) or MVBs where Aβ fibrils penetrate the vesicular membrane (*Lower*). Assignments of other vesicular structures (V) and cytoplasm (Cyt) shown. Arrows show fibrillar Aβ bundles. The MVB of the bottom image is significantly deformed from an ideal elliptical shape, possibly indicating the presence of stiff material inside the vesicle lumen, such as amyloid fibrils. Immunogold-labeling of Aβ after plaque formation in J774A.1 cells.