Expanded View Figures

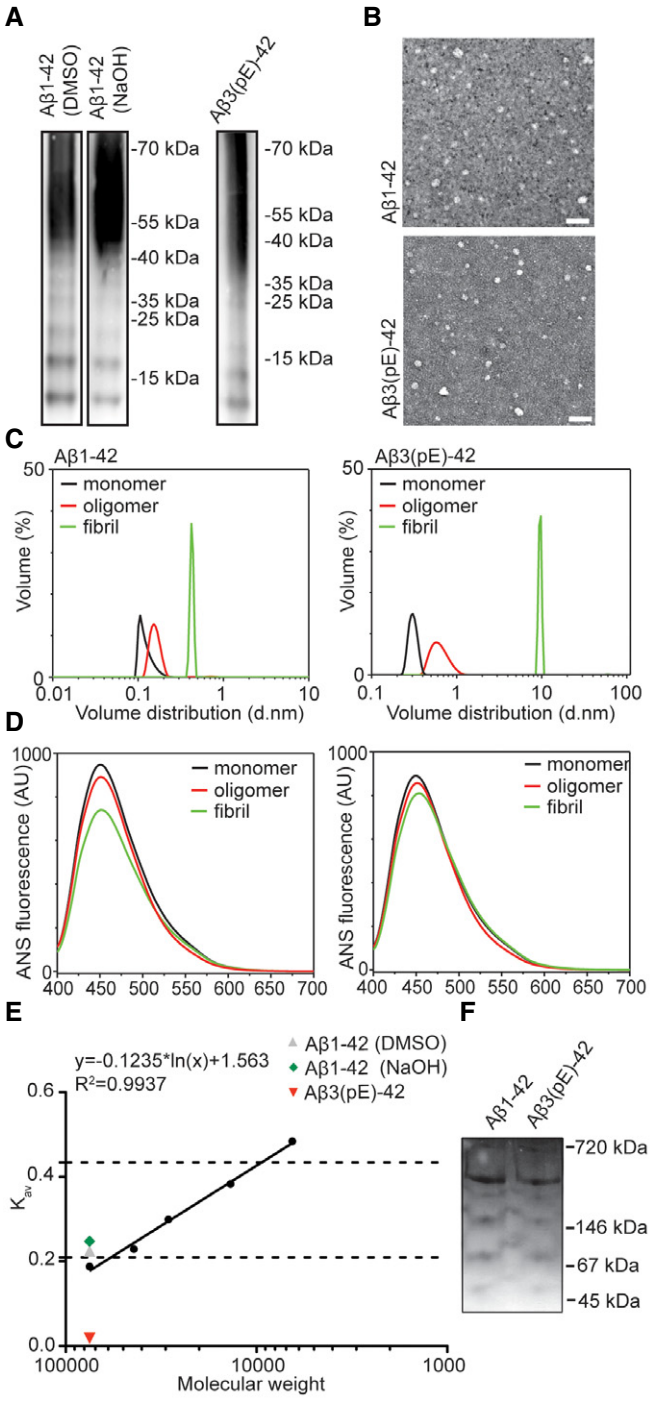

Figure EV1. Characterisation of oligomeric preparation.

A SDS–PAGE revealed a similar distribution of Aβ3(pE)-42 and Aβ1-42 species prepared with two different protocols with low (from 12 to 65 kDa) and higher molecular weight oligomers. The oligomerisation method does not significantly influence the distribution of Aβ1-42.

B Images from TEM revealed globular but not fibrillar structures for both peptide preparation. Scale bar, 50 nm.

C Volume distribution of monomeric (black line), oligomeric (red line) and fibrillar (green line) preparations measured by dynamic light scattering shows distinct size distributions for all preparations.

D ANS spectroscopy measurements revealed different surface hydrophobicity for monomeric (black line), oligomeric (red line) and fibrillar (green line) preparations of both peptides.

E Graph representing gel filtration column calibration and the highest amount of eluted oligomers detected by an ELISA assay. Dashed lines indicate low-n oligomer range (12–65 kDa).

F Image of native PAGE gel reveals that both preparations have similar migration pattern.

Source data are available online for this figure.

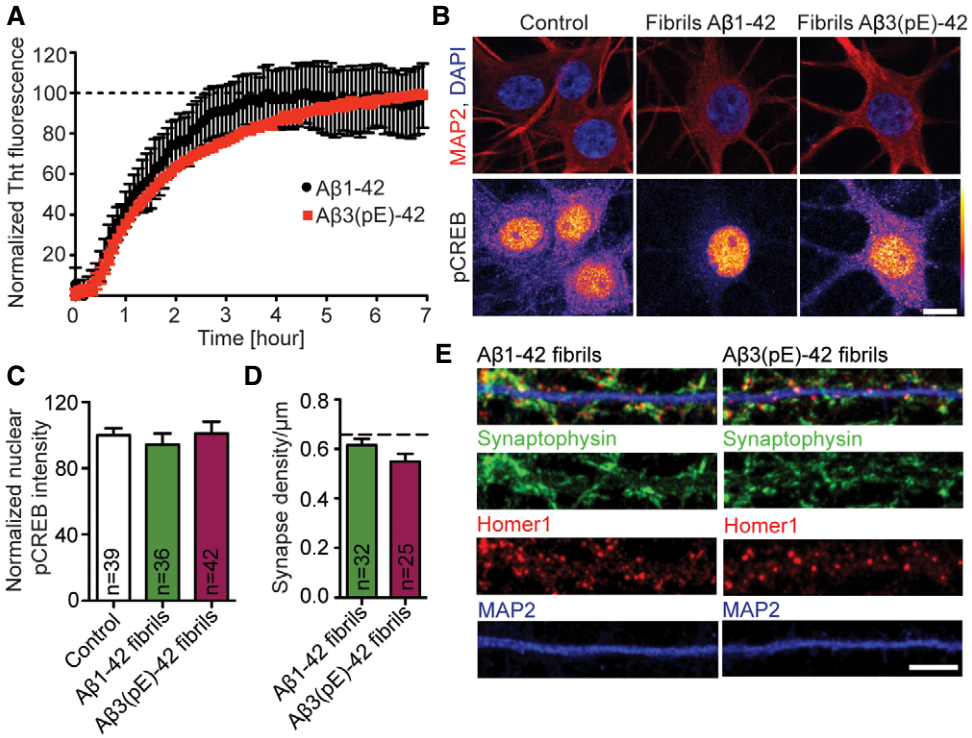

Figure EV2. Fibrilar forms of Aβ1-42 or Aβ3(pE)-42 do not cause significant synapse loss and CREB shut-off.

A Graph showing fibrillisation kinetics of Aβ1-42 (n = 3) and Aβ3(pE)-42 (n = 2). The fluorescence increases over time until reaching a plateau (dashed line) indicating formation of stable fibrils used in further experiments.

B, C Treatment with Aβ1-42 fibrils and Aβ3(pE)-42 fibrils do not alter pCREB nuclear levels. (B) Confocal images averaged from two sections of the nucleus of DIV18, primary, hippocampal neurons stained for MAP2, DAPI and pCREB. Original pixel intensities from 0 to 255 are represented as a gradient lookup table. Scale bar, 10 µm. (C) Bar graph representing mean nuclear pCREB staining intensity. n corresponds to the number of nuclei from different neurons analysed from at least four independent coverslips and at least two independent cell cultures.

D, E Neither Aβ1-42 nor Aβ3(pE)-42 fibrils cause synaptic loss. (D) Bar plot representing synaptic density of DIV20 neurons stained for Synaptophysin, Homer1 and MAP2 after Aβ1-42 and Aβ3(pE)-42 treatment compared to not treated control, represented in Fig 1B. Dashed line indicates mean control synapse density from Fig 1B. n corresponds to the number of separate dendritic segments on different neurons analysed from at least three independent coverslips and at least two independent cell cultures. (E) Representative confocal images of dendrites used for quantification. Scale bar, 5 µm.

Data information: P-values versus control by one-way ANOVA. Data are presented as the mean ± s.e.m.

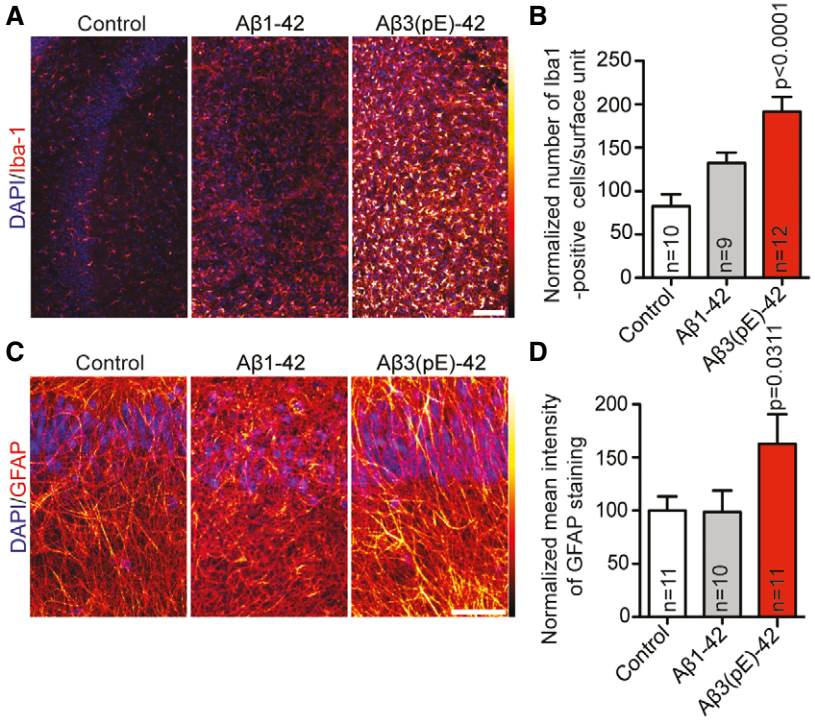

Figure EV3. Aβ3(pE)-42 causes prominent microglia and astroglia activation.

A–D Murine organotypic slices were treated with Aβ1-42 or Aβ3(pE)-42 for 3 days. Confocal images of slices stained for (A) Iba-1 or (C) GFAP indicate that there is an increased activation of microglia and astrocytes. Scale bar, 100 μm. Bar graphs show quantification of (B) Iba-1-positive cells and (D) mean GFAP fluorescence intensity. Original pixel intensities from 0 to 255 are represented as a gradient lookup table. Scale bar, 10 μm. *n* corresponds to the number of slices from at least two independent experiments. *P*-values versus control by one-way ANOVA. Data are represented as mean ± s.e.m.

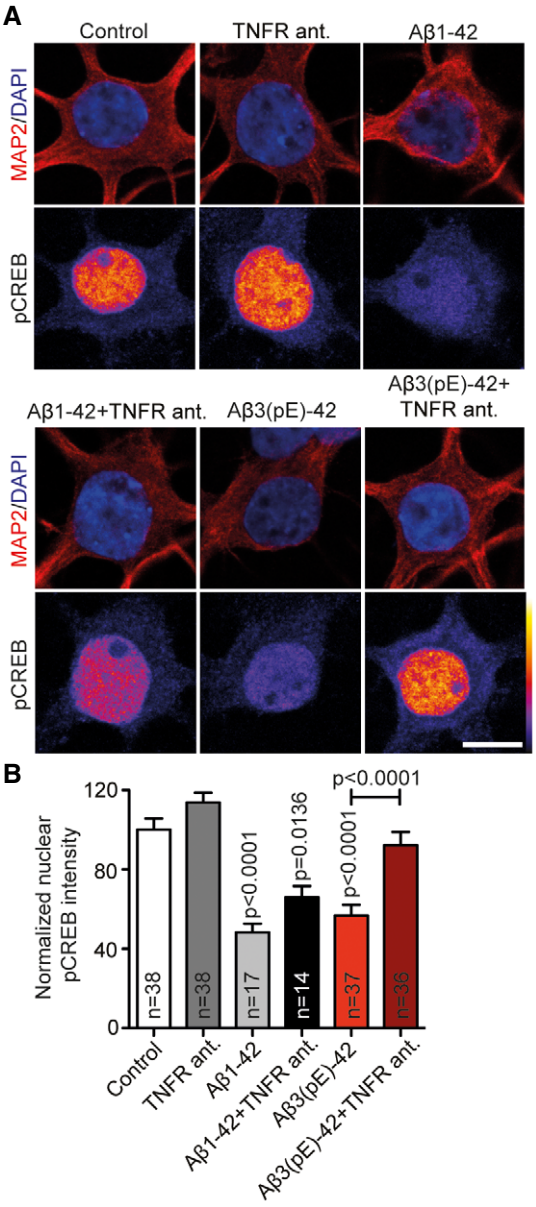

Figure EV4. Co-application of TNFR antagonist rescues Aβ3(pE)-42-caused decrease in pCREB.

A Confocal images averaged from two sections of the nucleus of DIV18 primary hippocampal neurons stained for MAP2, DAPI and pCREB (Ser133) treated with Aβ1-42 or Aβ3(pE)-42 for 40 min with or without the TNFR antagonist. Original pixel intensities from 0 to 255 are represented as a gradient lookup table. Scale bar, 10 μm.

B Only in case of Aβ3(pE)-42 co-application of the TNFR antagonist prevents CREB shut-off. *n* corresponds to the number of nuclei from different neurons analysed from at least four independent coverslips from two independent cell cultures. *P*-values versus control by one-way ANOVA. Data are represented as mean ± s.e.m.

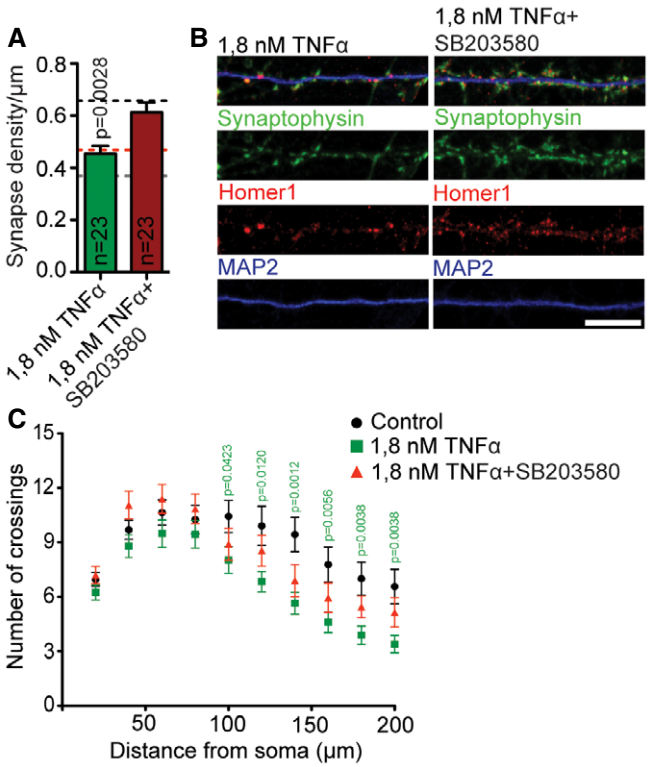

Figure EV5. 1.8 nM TNFα causes synapse loss and reduction in dendritic tree complexity.

A Bar plots representing mean synapse density after treatment with 1.8 nM TNFα with or without co-application of SB203580. 1.8 nM TNFα causes synaptic loss that can be rescued by SB203580 application. Dashed lines indicate mean synapse density from Fig 1B: black, control; grey, Aβ1-42 treatment; red, Aβ3(pE)-42 treatment. *n* corresponds to the number of different dendritic segments from different neurons analysed from at least four independent coverslips and at least two independent cell cultures.

B Confocal images of representative dendrites stained for Homer1, Synaptophysin and MAP2. Scale bar, 10 µm.

C 1.8 nM TNFα causes dendritic tree simplification that can be rescued by SB203580 application. Graph representing results of Sholl analysis of RFP-transfected neurons treated with 1.8 nM TNFα (n = 25) with or without SB203580 (n = 20) and control (n = 23). *n* corresponds to the number of neurons analysed from at least three independent coverslips and at least two independent cell cultures.

Data information: *P*-values refer to control by one-way ANOVA. For Sholl analysis, two-way ANOVA followed by an unpaired two-tailed Student's *t*-test was used. *P*-values in green refer to TNFα compared to control. Data are represented as mean ± s.e.m.