Appendix

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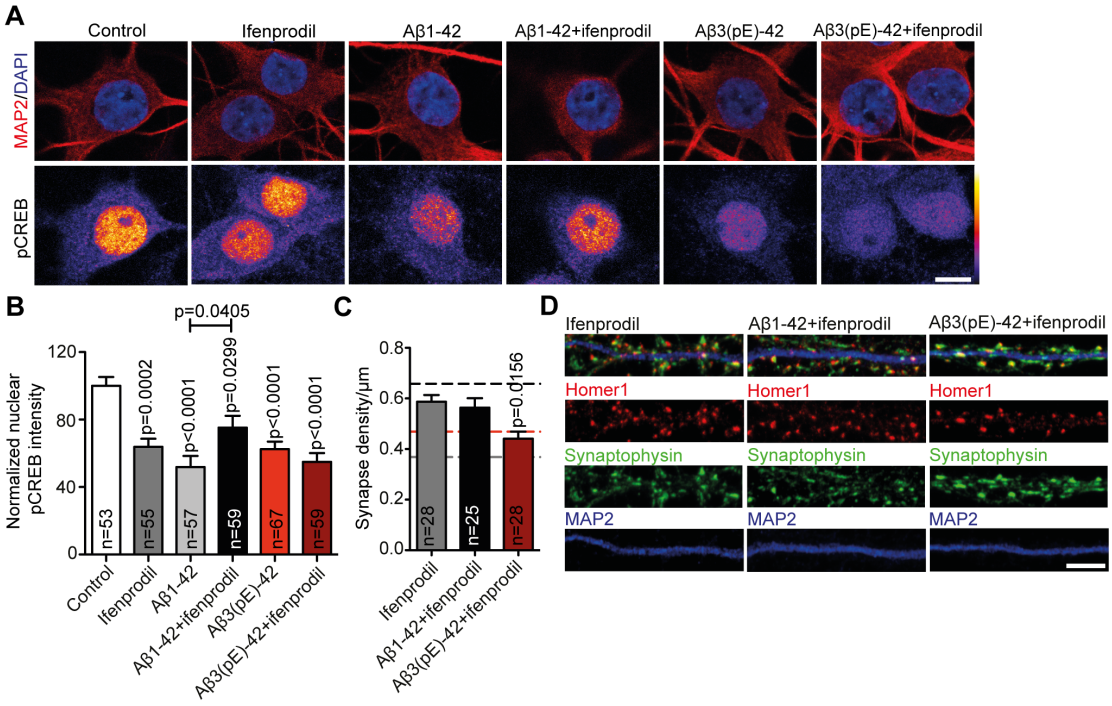
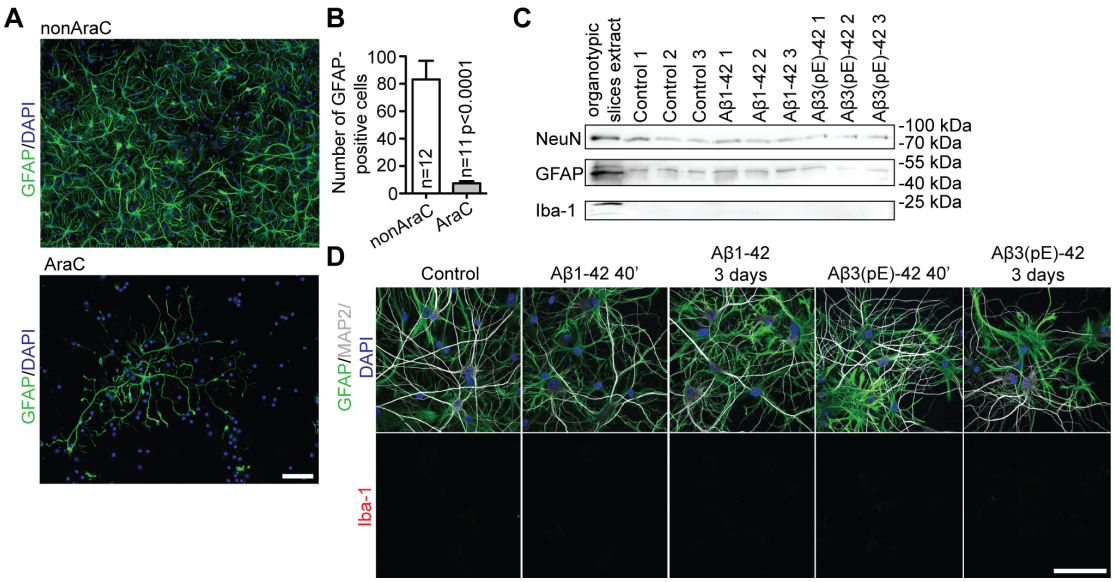
Figure S1.



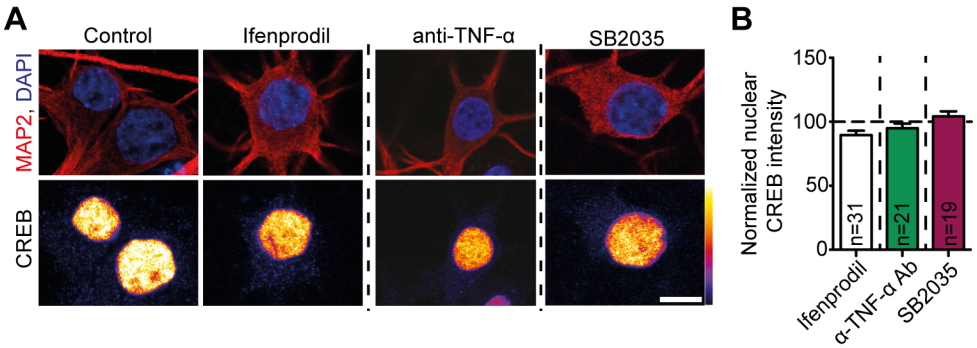
Appendix Figure S1: The GluN2B antagonist ifenprodil prevents Aβ1-42- but not Aβ3(pE)-42- induced CREB shut-off and synapse loss. (**A**) Images of representative neurons. Original pixel intensities from 0 to 255 are represented as a gradient lookup table. Scale bar 10 µm. (**B**) Although ifenprodil *per se* decreases nuclear pCREB intensity, it partially rescues Aβ1-42-caused CREB shut-off. N corresponds to the number of nuclei analysed from at least 3 independent coverslips and at least 2 independent cell cultures. (**C**) Incubation with ifenprodil rescues Aβ1-42- but not Aβ3(pE)-42-induced synaptic loss. Dashed lines indicate mean synapse density from Fig 1B (black, grey and red for control, Aβ1-42 and Aβ3(pE)-42, respectively). N corresponds to the number of different dendritic segments from different neurons analysed from at least 4 independent coverslips and at least 2 independent cell cultures (**D**) Confocal images of representative dendrites stained for Homer1, Synaptophysin and MAP2. Scale bar is 5 µm. P-values versus control by one-way ANOVA. Data are represented as mean ± s.e.m.

Figure S2.



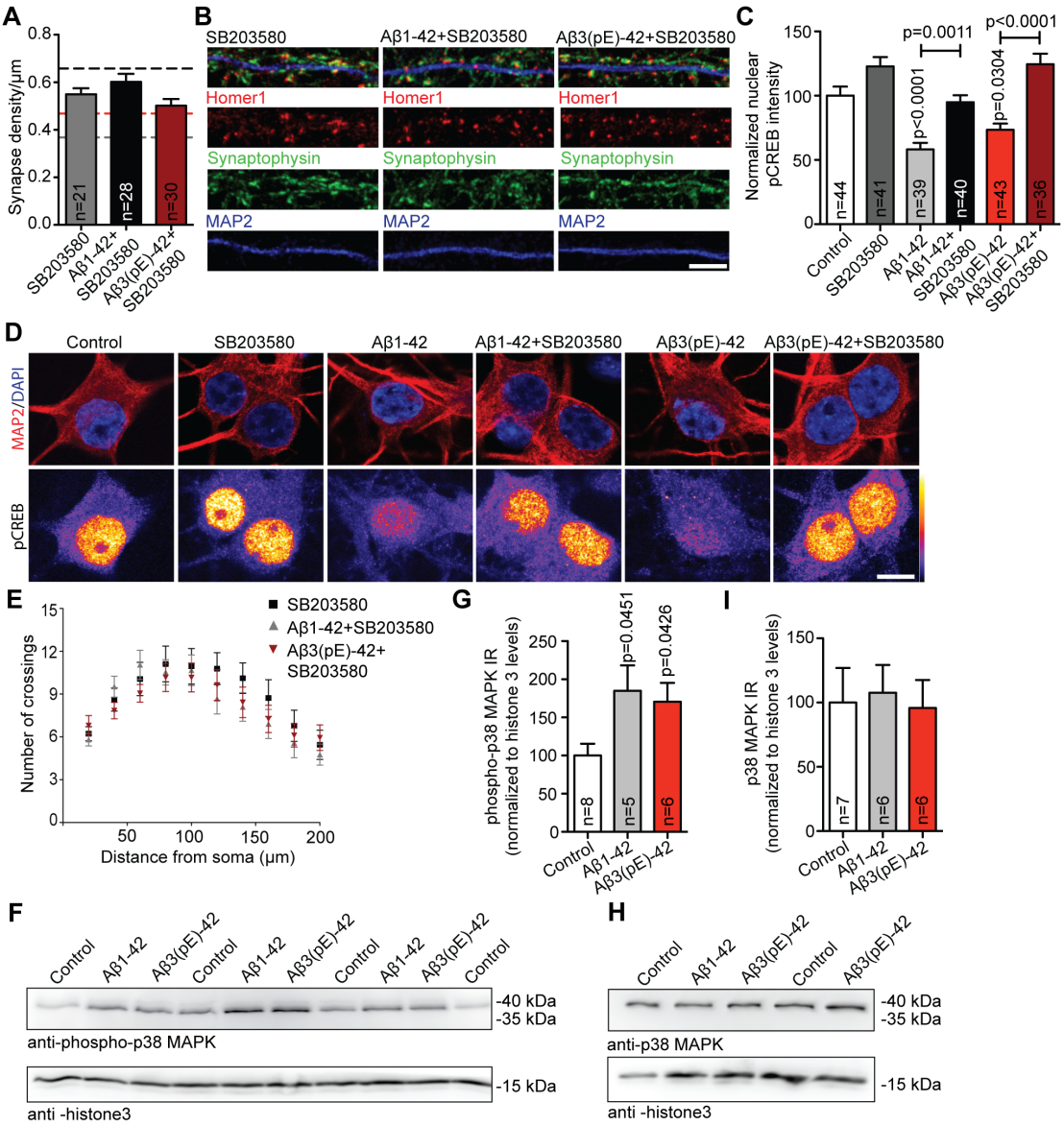
Appendix Figure S2: Application of AraC to neuronal culture causes significant reduction in astroglia content. (**A**) Representative fluorescent microscope images of DIV18 cultures stained for GFAP and DAPI. Scale bar is 100 μm. (**B**) Bar plot representing number of astrocytes per field of view (ROI). P-values versus control by two-tailed Student t-test. N corresponds to the number of ROIs from 2 independent cell cultures. Data are presented as the mean ± s.e.m. (**C**) SDS-PAGE of organotypic slices extract (positive control) or cell culture extracts treated with oligomers for 3 days. Membrane was probed with antibodies detecting Iba-1, GFAP and NeuN. 1, 2, 3 indicate independent cultures from which extracts were prepared. (**D**) Representative confocal images of cultures stained with Iba-1, GFAP and NeuN. The cells were untreated (control) or treated with oligomers for 40 min or 3 days. Cultures contained neurons (MAP2) and astrocytes (GFAP). No Iba-1 positive signal was detected (contrary to organotypic slices, EV3). Scale bar is 100 μm.

Figure S3.



Appendix Figure S3: 40 min treatment with ifenprodil, anti-TNFα antibody (Ab), astrocyte conditioned media or SB203580 does not alter nuclear CREB levels. (**A**) Confocal images averaged from two sections of the nucleus of DIV18, primary hippocampal neurons stained for MAP2, DAPI and CREB. Original pixel intensities from 0 to 255 are represented as a gradient lookup table. Scale bar is 10 µm. (**B**) Bar plot representing average CREB nuclear staining intensity. The experiments were conducted in parallel to the results represented in (**A, B**) Ifenprodil panel - Figure S1A and S1B; anti-TNFα Ab panel Figure 8Cc and 8D; SB203580 panel – Figure S4C and S4D. Dashed line indicates that separate panels were not conducted together but normalized to control with each individual experiment. N corresponds to the number of nuclei analysed from at least 3 independent coverslips and at least 2 independent cell cultures. P-values versus control by one-way ANOVA. Data are presented as the mean ± s.e.m.

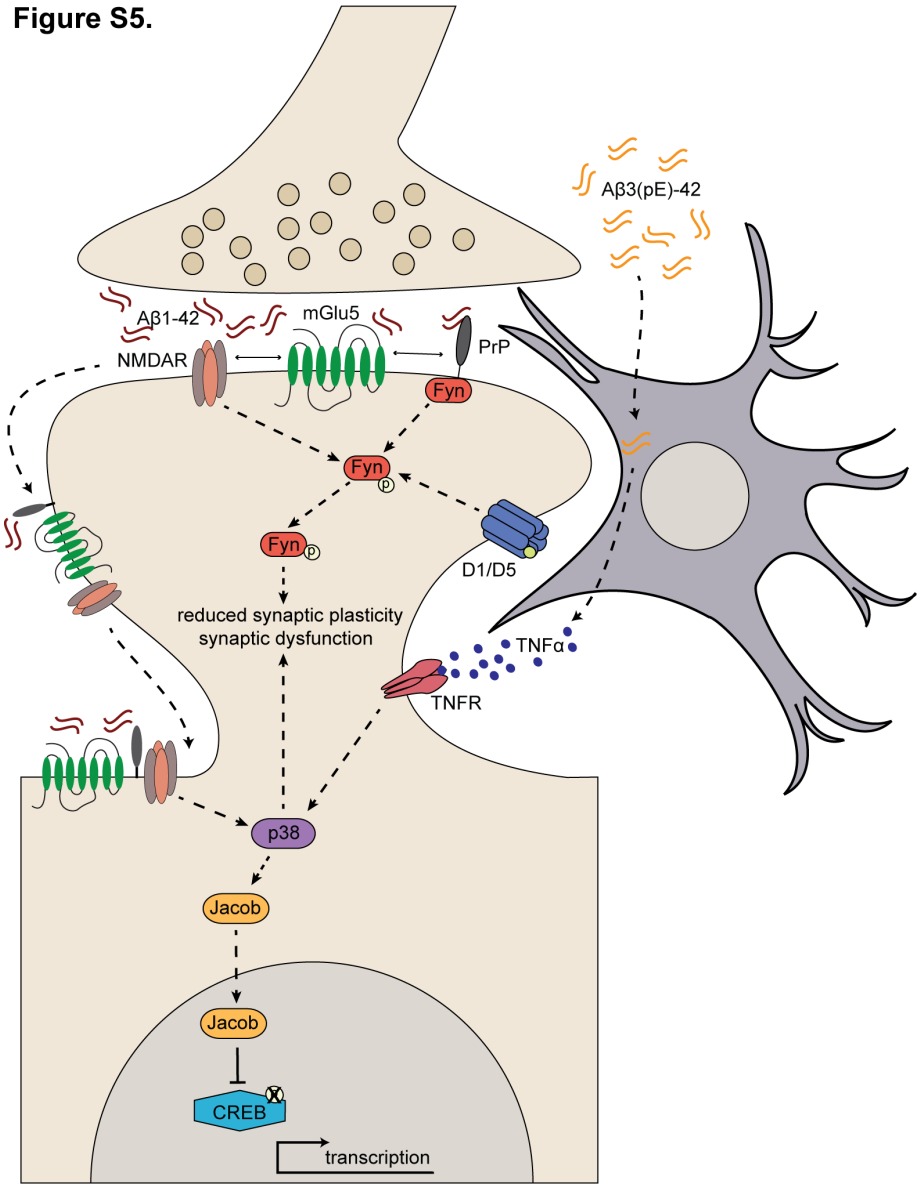
Figure S4.



Appendix Figure S4: The p38MAPK inhibitor SB203580 rescues Aβ1-42- and Aβ3(pE)-42-induced synapse loss, dendrite retraction and CREB shut-off. (**A**) SB203580 causes decrease in synaptic density and abolishes synaptic loss after treatment with Aβ1-42 and Aβ3(pE)-42. Bar plot representing mean synaptic density. Dashed lines indicate mean synapse density from Fig 1B: black - control, grey -Aβ1-42 treatment and red - Aβ3(pE)-42 treatment. N corresponds to the number of different dendritic segments from different neurons analysed from at least 4 independent coverslips and at least 2 independent cell cultures (**B**) Confocal images of representative dendrites stained for Homer1, Synaptophysin and MAP2. Scale bar is 5 µm. (**C**) Pre-treatment with p38MAPK inhibitor prevents Aβ oligomers-caused CREB shut-off. Bar graph representing mean nuclear pCREB staining intensity. N corresponds to the number of nuclei analysed from at least 3 independent coverslips and at least 2 independent cell cultures. (**D**) 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 is 10 μm. (**E**) Co-application of SB203580 rescues both Aβ1-42- and Aβ3(pE)-42-induced dendritic tree simplification. N corresponds to the number of neurons (SB203580 n=18, SB203580+Aβ1-42 n=21, SB203580+Aβ3(pE)-42 n=19) analysed from at least 3 independent coverslips and at least 2 independent cell cultures. (**F**) SDS-PAGE revealed that both Aβ3(pE)-42 and Aβ1-42 cause activation of p38 MAPK. Representative images of immunoblot of neuronal extracts probed with anti-phospho-p38 MAPK antibody and Histone 3. (**G**) Bar plot representing quantification of phosphor-p38 MAPK immunoblot of neuronal extracts treated with Aβ3(pE)-42 or Aβ1-42. N corresponds to the number of wells from which extracts were prepared from 2 independent cell cultures. (**H**) SDS-PAGE revealed that application of both Aβ3(pE)-42 and Aβ1-42 does not change p38 MAPK protein levels. Representative images of immunoblot of neuronal extracts probed with p38 MAPK antibody and Histone 3. (**I**) Bar plot representing quantification of p38 MAPK immunoblot of neuronal extracts treated with Aβ3(pE)-42 or Aβ1-42. N corresponds to the number of wells from which extracts were prepared from 2 independent cell cultures. For Sholl analysis two-way ANOVA followed by an unpaired two-tailed Student t-test was used. P-values versus control by one-way ANOVA. Data are presented as the mean ± s.e.m. For statistical analysis immunoblot values a Mann Whitney U-test was used.

Figure S5.



Appendix Figure S5: Aβ1-42 and Aβ3(pE)-42 impair synaptic plasticity and synaptic dysfunction via distinct mechanisms. Aβ1-42 interacts directly with synaptic receptors, possibly causing a switch from synaptic to extrasynaptic signalling. It seems possible that this mechanism is partially mediated by Fyn kinase, which regulates synaptic anchoring of NMDAR. Fyn kinase activation depends on dopamine D1/D5 receptors. In addition, it was shown that the Aβ1-42-triggered mGlu5-PrP abnormally activates Fyn kinase. Taken together the disturbance in synaptic vs extrasynaptic balance leads to the accumulation of non-phosphorylated Jacob in the nucleus and subsequent CREB shut-off. On the other hand, Aβ3(pE)-42 oligomers are taken up by astrocytes which causes release of pro-inflammatory cytokines like TNFα. Activation of a neuronal TNFα receptor leads to enhanced p38MAPK activity. p38MAPK activity could be a nodal point at which Aβ1-42 and Aβ3(pE)-42 neurotoxic function converge.