

Supplemental Figures

HDL Mimetic Peptide 4F Mitigates A β -Induced Inhibition of ApoE Secretion and Lipidation in Primary Astrocytes and Microglia

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Fig. S1

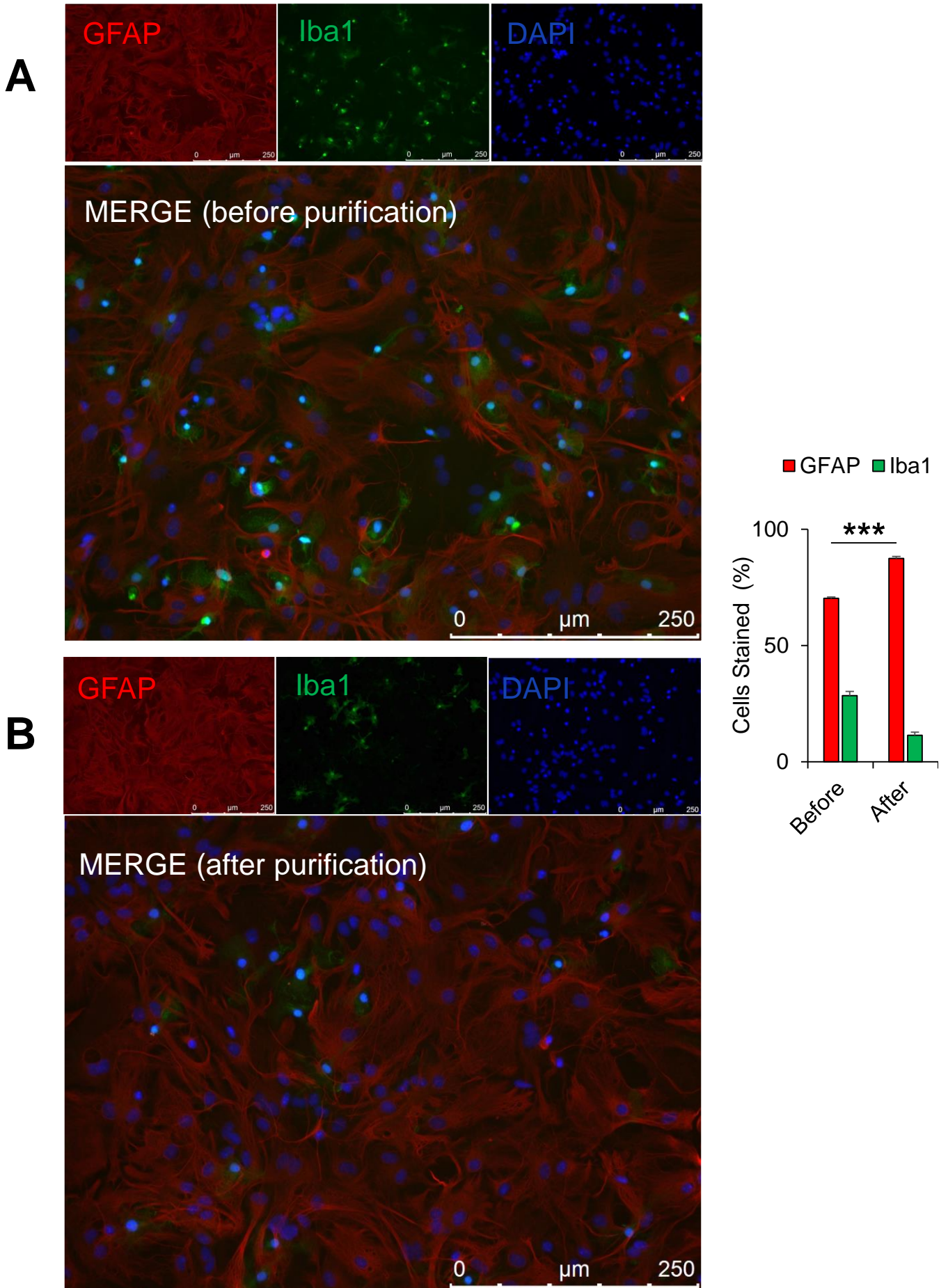


Fig. S1. Purification of primary mouse astrocyte cultures. Immunofluorescence analysis of markers for astrocytes (GFAP), microglia (Iba1), and the nuclei (DAPI) in mixed glial cultures before (A) and after (B) purification (n=3 cultures). GFAP – glial fibrillary acidic protein; Iba1 - ionized calcium-binding adapter molecule 1; DAPI - 4',6-diamidino-2-phenylindole. *** = $p < 0.001$.

Fig. S2

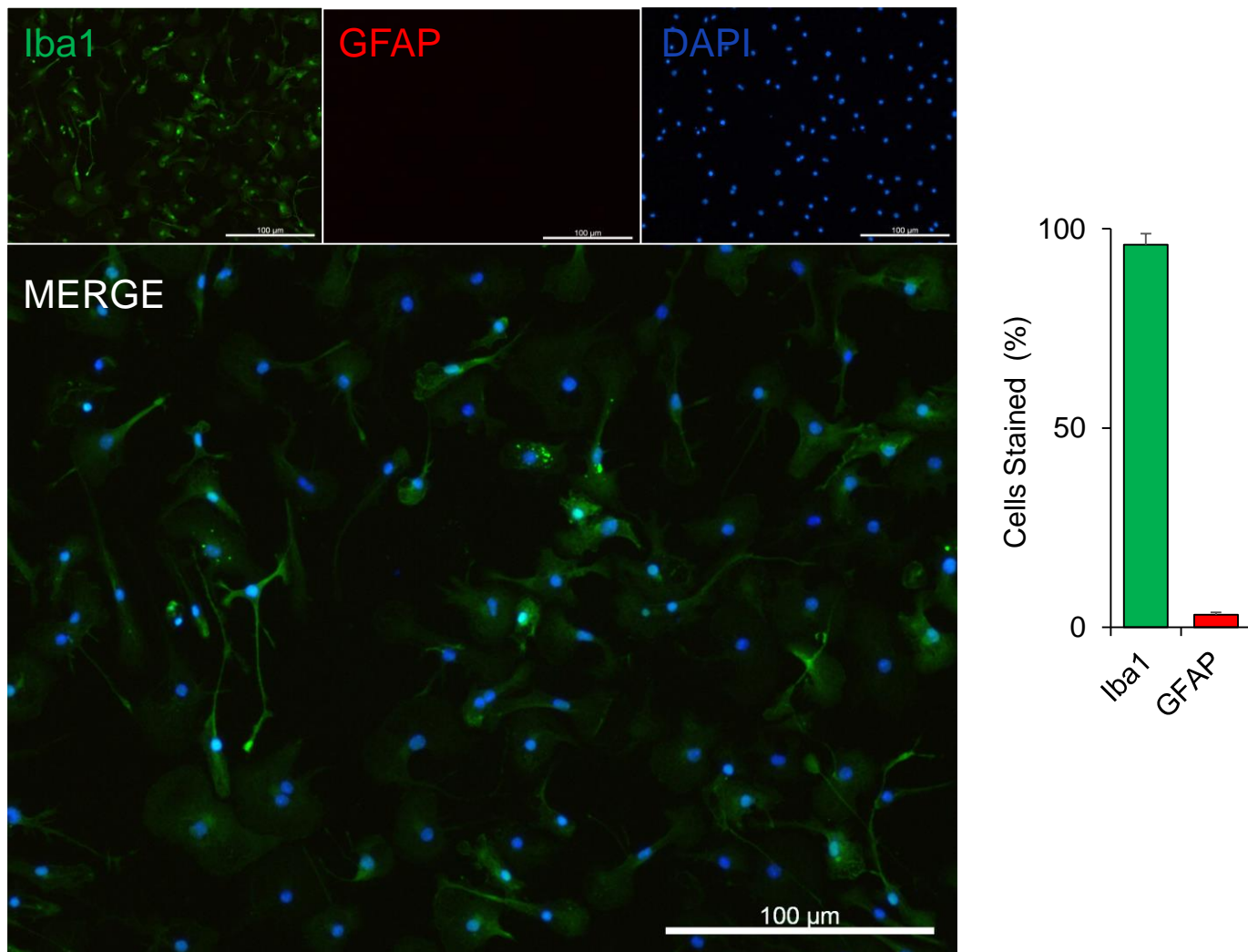


Fig. S2. Purity of primary mouse microglial cultures. Immunofluorescent analysis of markers for microglia (Iba1), astrocytes (GFAP), and the nuclei (DAPI) in purified primary mouse microglial cultures (n=5 cultures). Iba1 - ionized calcium-binding adapter molecule 1; GFAP – glial fibrillary acidic protein; DAPI - 4',6-diamidino-2-phenylindole.

Fig. S3

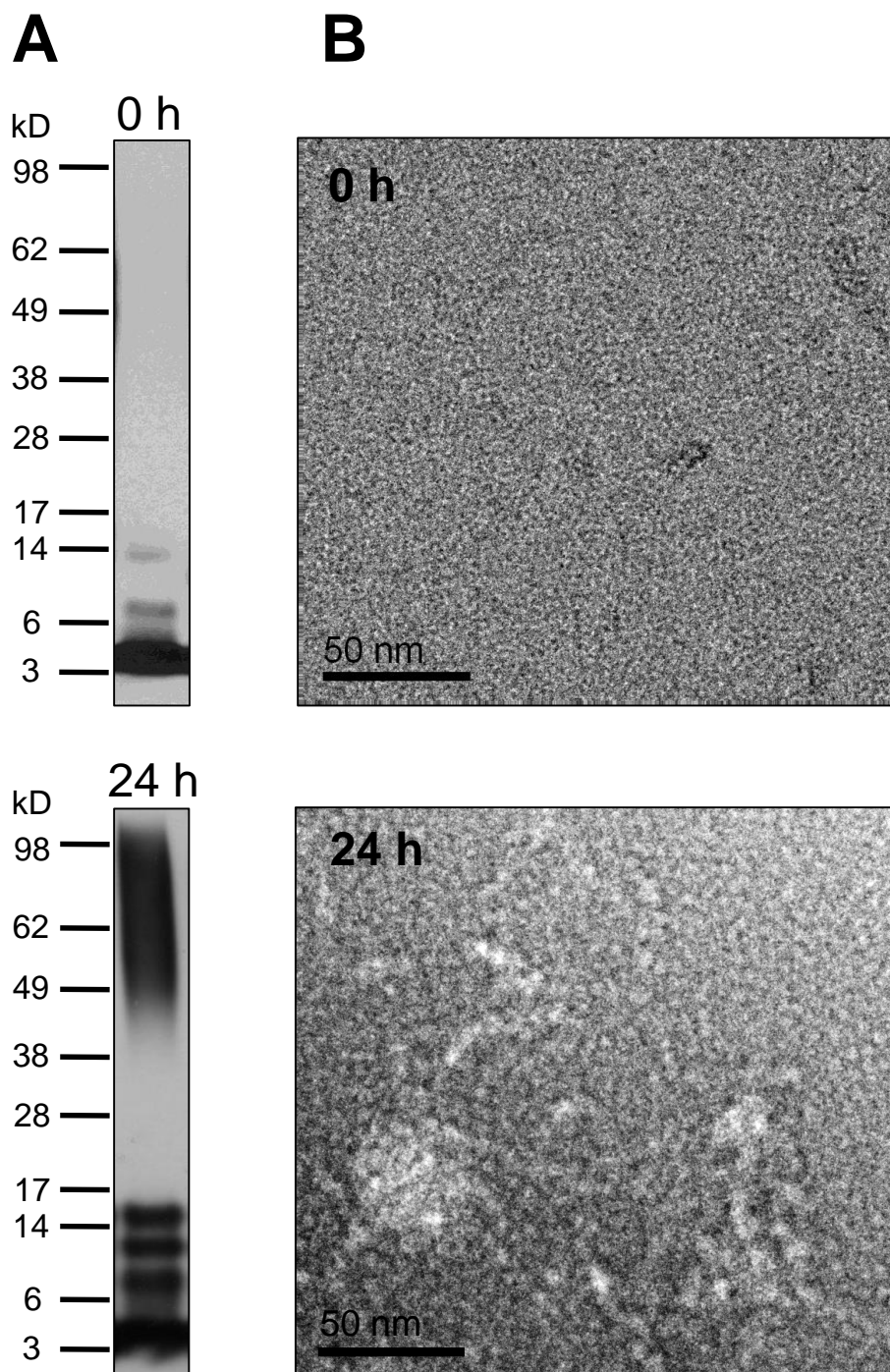


Fig. S3. Characterization of A β preparations. Representative images of Western blot (**A**) and transmission electron microscopy (TEM) (**B**) analysis of A β preparations (n=2) at time 0 and after 24 h incubation.

Fig. S4

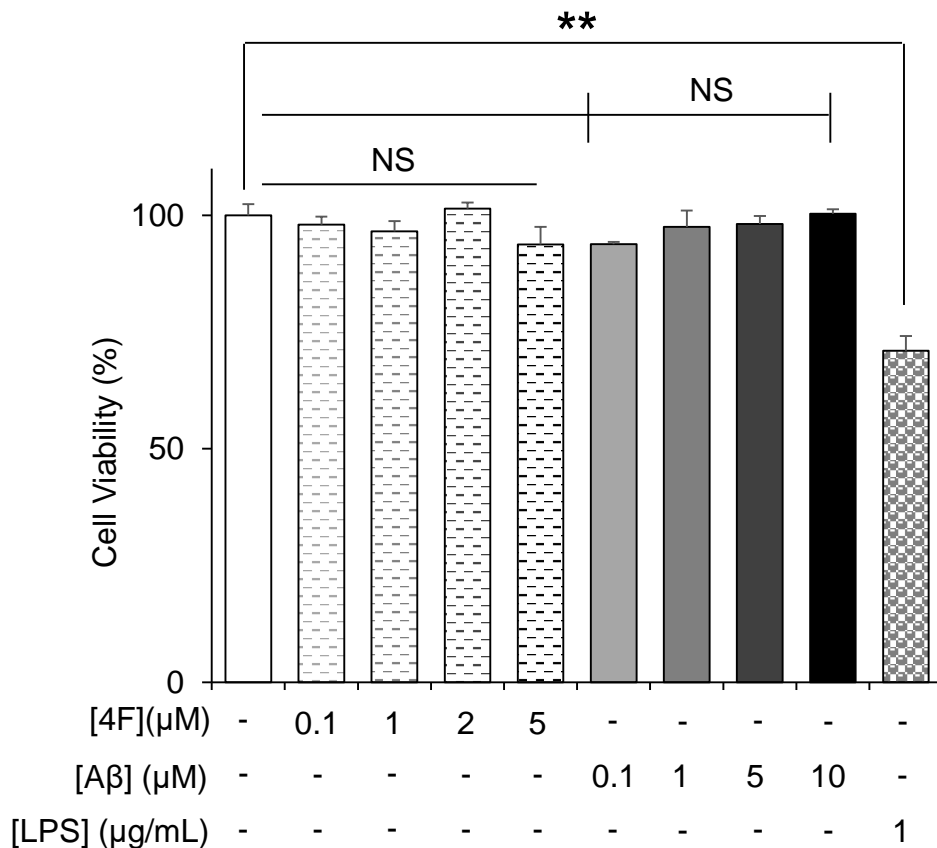


Fig. S4. 4F and A β treatment do not affect cell survival in primary mouse astrocyte cultures. The cultures were treated with different concentrations of 4F or A β for 24 h and followed by the CellTiter-Blue® Cell Viability Assay (Promega; Cat# G8080) (n=3 cultures). LPS was included as control. ** = $p < 0.01$; NS, not significant.

Fig. S5

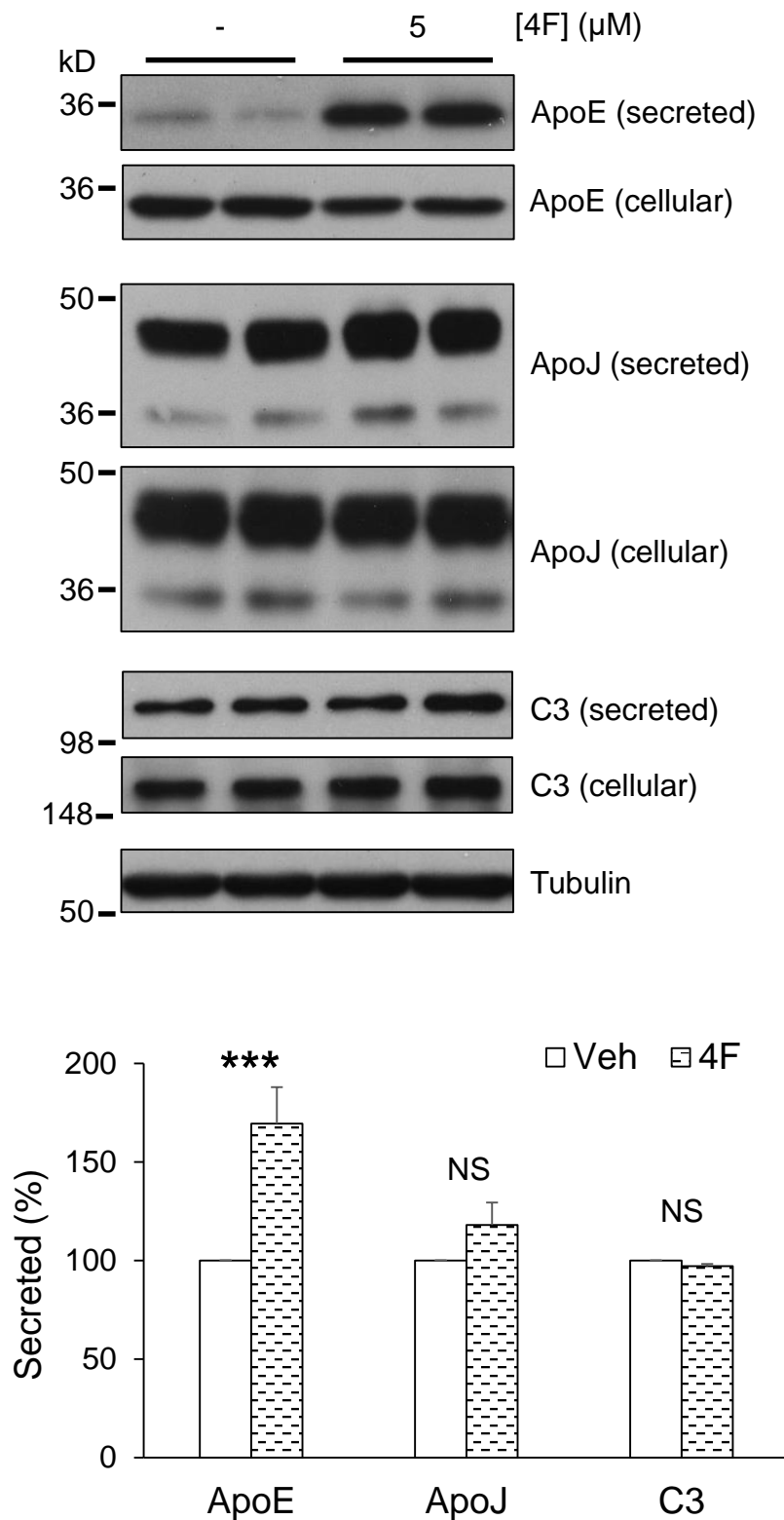


Fig. S5. Specific effects of 4F on apoE secretion in primary mouse astrocytes. Western blot analysis of apoE, apoJ, and complement C3 in conditioned media and cell lysates of primary astrocytes treated with or without 4F for 24h (n=3 independent experiments with each treatment in duplicate). *** = $p < 0.001$; NS, not significant.

Fig. S6

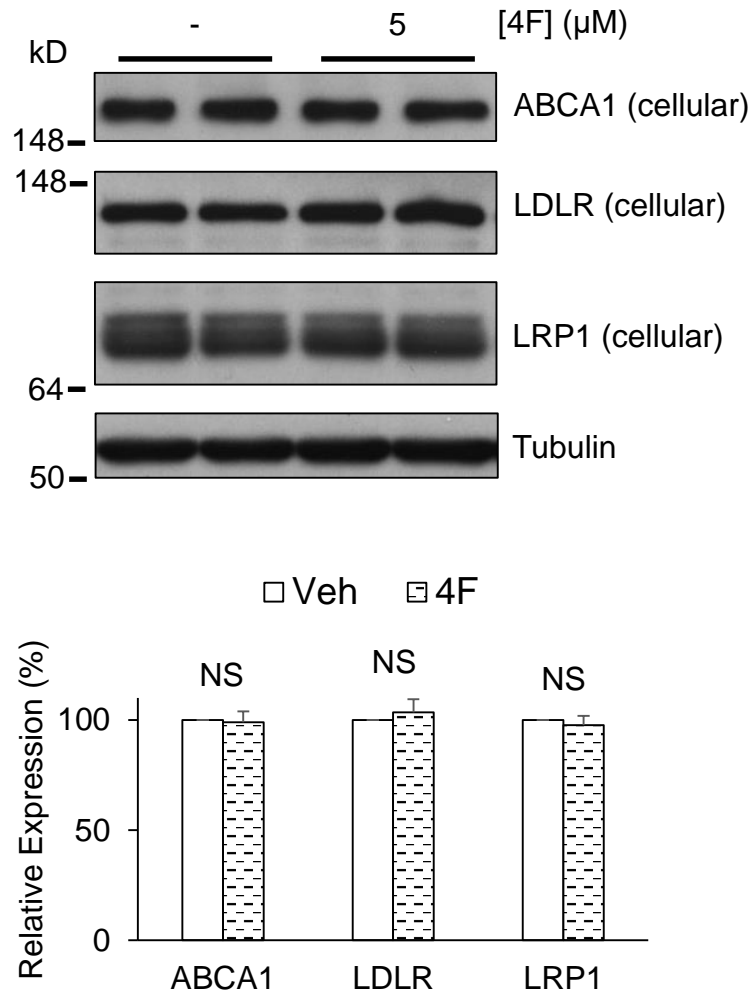


Fig. S6. 4F treatment does not affect the cellular levels of ABCA1, LDLR, and LRP1 in primary mouse astrocytes. Western blot analysis of ABCA1, LDLR, and LRP1 in cell lysates of primary astrocytes treated with or without 4F for 24h (n=3 independent experiments with each treatment in duplicate). Note that the same blots as in Fig. S5 were used to probe proteins in this figure. NS, not significant.

Fig. S7

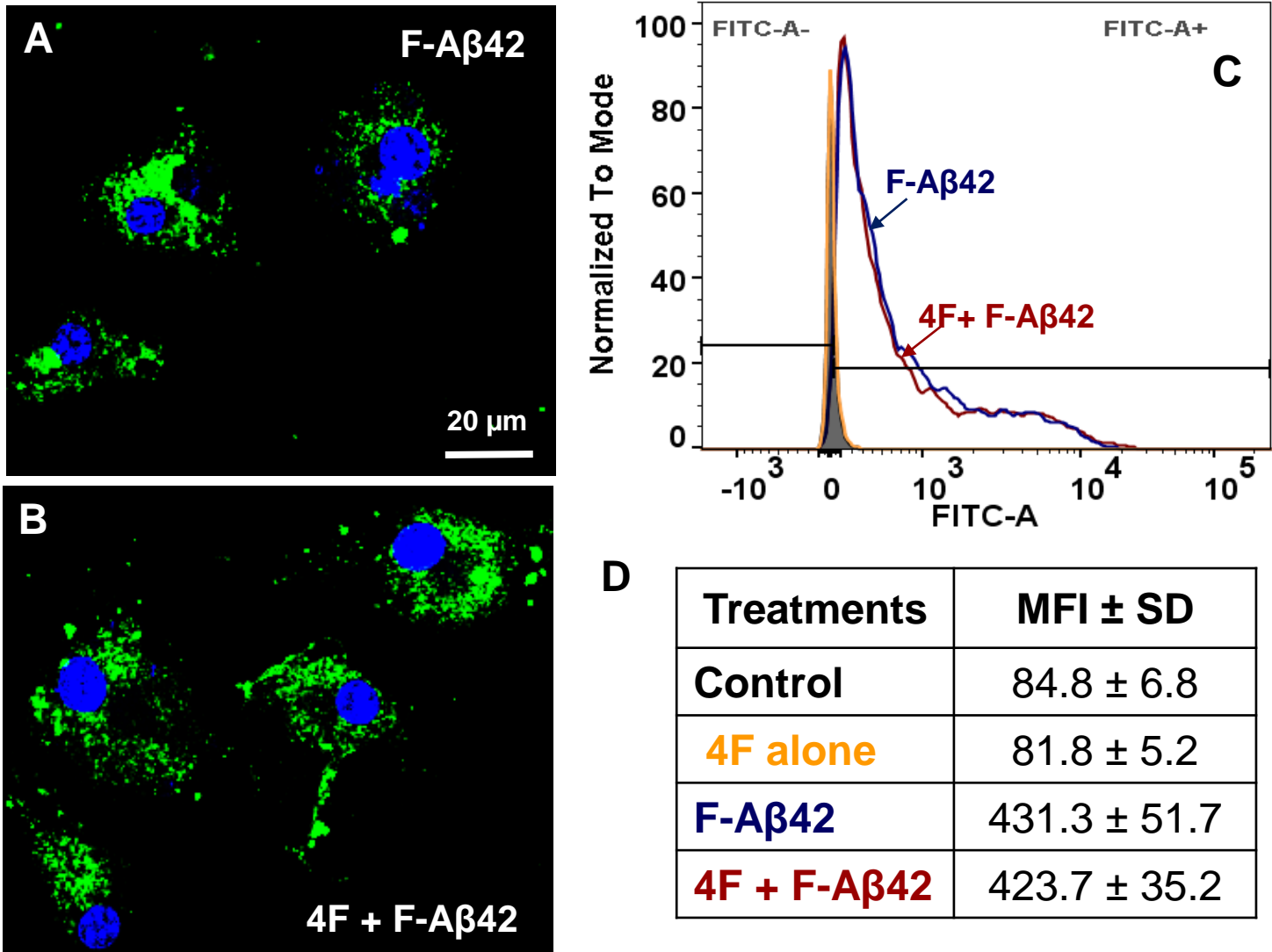


Fig. S7. 4F treatment does not affect the uptake of F-A β 42 in primary mouse astrocytes. Confocal microscopy (**A**, **B**) images and histograms from flowcytometry (**C**) of primary astrocytes treated with 1.6 μ M F-A β 42 alone (**A**) or in combination with 5 μ M 4F (**B**) for 1 h at 37 $^{\circ}$ C. Blue: DAPI, Green: F-A β 42. (**D**) Flowcytometry data analysis: FITC positive and negative cells were initially gated and then median fluorescence intensity (MFI) of FITC positive cells were obtained. Data is presented as average \pm SD (n= 3 cultures).