

# Neuroprotective Effects of Astaxanthin in Preclinical Models of Alzheimer's Disease

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## BACKGROUND

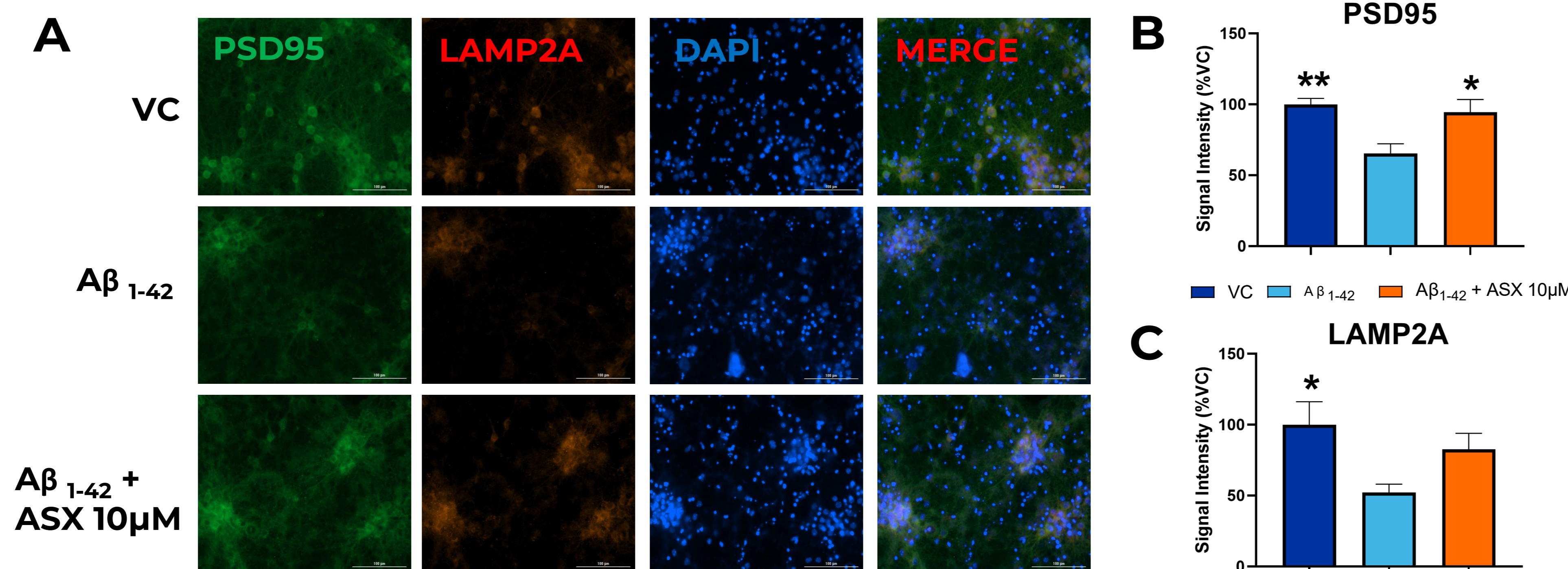
Alzheimer's disease (AD) is a debilitating neurodegenerative disease with a global population burden projected to triple by 2050. AD is the most common cause of dementia, and it accounts for about 70% of all dementia cases. The pathological manifestations of Alzheimer's disease include aggregation of misfolded protein fragment beta-amyloid outside neurons called plaques, hyper-phosphorylation of protein tau inside neurons called tangles, neuronal loss, synaptic degeneration, and neuroinflammation. Until date, there are no disease-modifying therapies available. Astaxanthin, a lipid-soluble xanthophyll beta-carotenoid synthesized by many microorganisms, has been reported to exhibit anti-inflammatory and neuroprotective functions. In this study we investigated the effects of astaxanthin on synaptic degeneration, autophagy, neuronal loss and neuroinflammation as pathological hallmarks of AD using two cell culture models. Our data suggest astaxanthin to be a good candidate for the treatment of AD, although further analyses of its *in vivo* effects have to be performed to validate observed *in vitro* results.

## MATERIALS AND METHODS

Primary mouse hippocampal neurons and organotypic brain slice cultures were treated with either amyloid beta ( $A\beta_{1-42}$ ) or lipopolysaccharides (LPS) and co-incubated with astaxanthin (ASX) or dexamethasone (DEXA). DEXA is an anti-inflammatory agent used as reference substance. Expression levels of relevant markers were examined on mRNA level by quantitative real time polymerase chain reaction (qRT-PCR) and immunofluorescent labeling techniques. Cytokine release was determined using an immunosorbent assay (MesoScale Discovery).

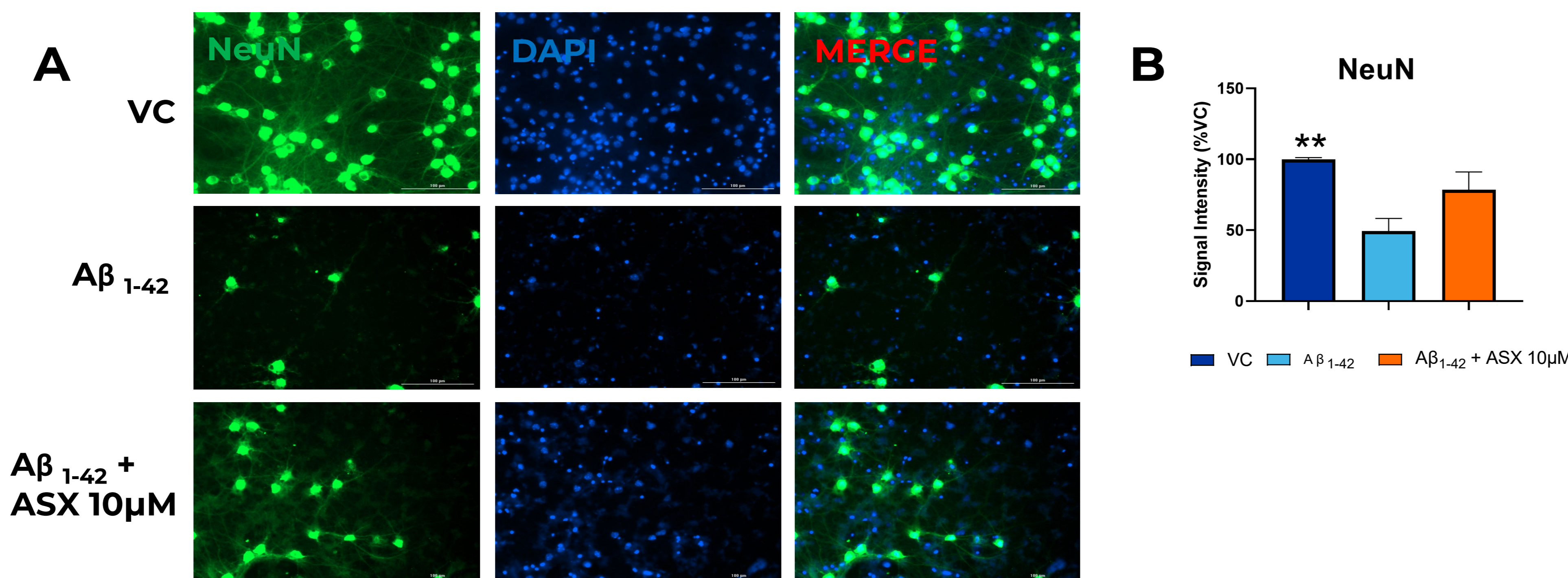
## RESULTS

### Astaxanthin Increases PSD95 and Effects LAMP2A Signaling in $A\beta_{1-42}$ Treated Primary Hippocampal Neuronal Cells



**Figure 1. Effect of astaxanthin (ASX) on  $A\beta_{1-42}$  induced toxicity in hippocampal neurons.** Representative images (A) and signal quantification of post synaptic (PSD95) and chaperone-mediated autophagy (LAMP2A) markers (B,C) in vehicle,  $A\beta_{1-42}$  and  $A\beta_{1-42}$  in combination with ASX treated primary hippocampal neurons. One-Way ANOVA with Dunnett's multiple comparisons test vs.  $A\beta_{1-42}$ . Mean + SEM; n=6-12. \*p<0.05, \*\*p<0.01.

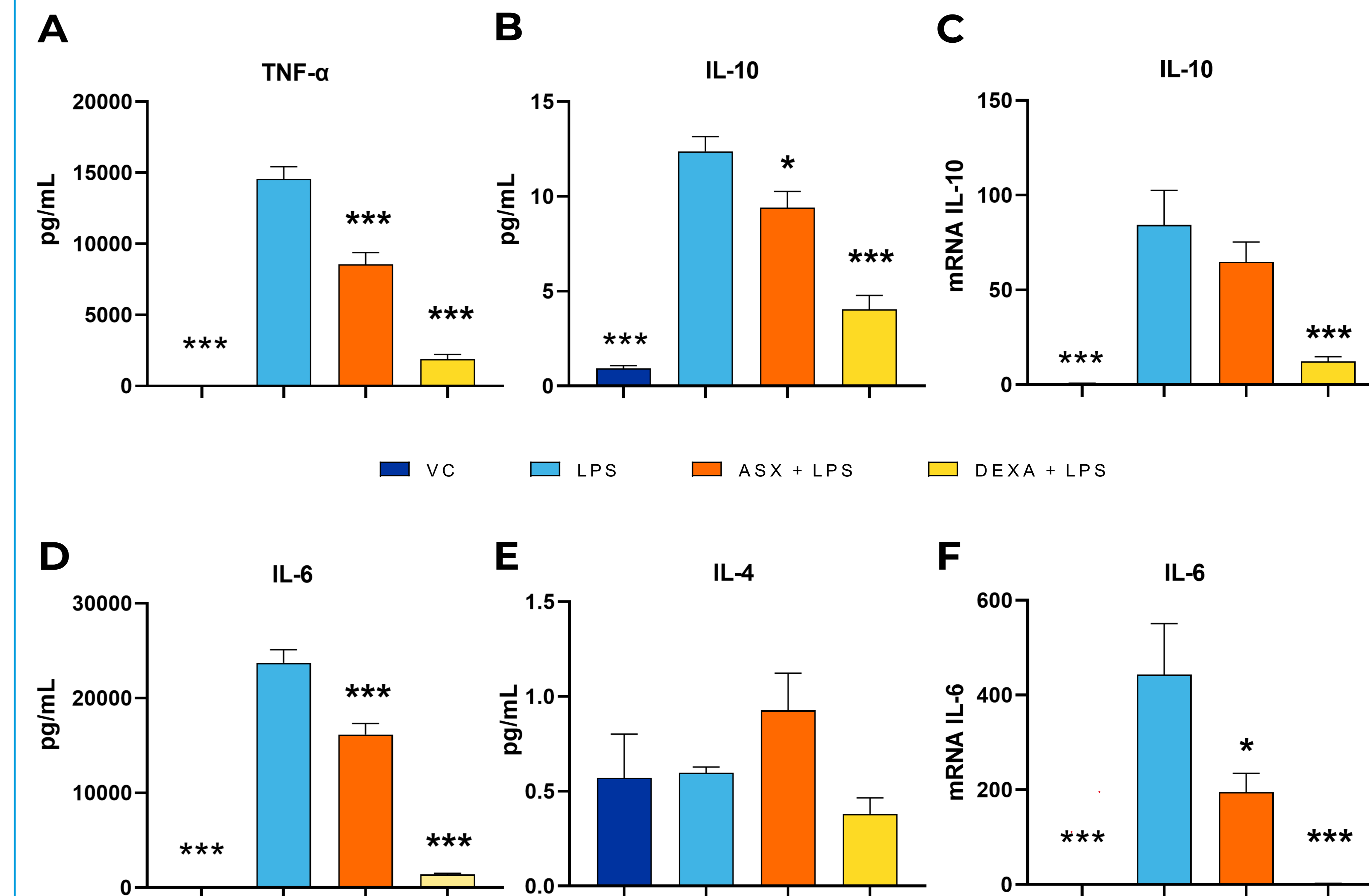
### Astaxanthin Partially Rescues Neuronal Loss in $A\beta_{1-42}$ Treated Primary Hippocampal Neuronal Cells



**Figure 2. Effect of astaxanthin (ASX) on  $A\beta_{1-42}$  induced toxicity in hippocampal neurons.** Representative images (A) and signal quantification (B) of neuronal marker (NeuN) labelling in vehicle,  $A\beta_{1-42}$  and  $A\beta_{1-42}$  in combination with ASX-treated primary hippocampal neurons. One-Way ANOVA with Dunnett's multiple comparisons test vs.  $A\beta_{1-42}$ . Mean + SEM; n=6. \*\*p<0.01

## RESULTS

### Astaxanthin Reduces the Secretion of Cytokines in LPS-stimulated Organotypic Hippocampal Brain Slices



**Figure 3. Effect of astaxanthin (ASX) on cytokine release in lipopolysaccharide (LPS)-stimulated brain slices.** Organotypic hippocampal brain slices were incubated with 10 ng/ mL LPS as well as with 10 ng/mL LPS in combination with 50  $\mu$ M ASX or 10  $\mu$ M dexamethasone (DEXA) for 24 h followed by detection of cytokine release into the supernatant by MSD and mRNA quantification of relevant pro-inflammatory markers by qRT-PCR. One-Way ANOVA with Dunnett's multiple comparisons test vs. LPS. Mean + SEM; n=5-9. \*p<0.05, \*\*\*p<0.001.

## CONCLUSION

Our data suggest that astaxanthin might be a therapeutic candidate by ameliorating pathophysiological manifestations associated with Alzheimer's disease.