S1. Supplementary Results and Discussion

S1.1. Other proinflammatory agents not affected by antagonizing Aβ•CaSR signaling

In keeping with previous works from other Laboratories [1-3], our results show that Aβ-exposed NAHAs overproduce and overrelease a set cytokines and chemokines, i.e. IL-1β, IL-3, IL-8, IL-16, the secretion of which NPS 2143 did not hinder [Table 1; Figure S1]. All these cytokines/chemokines have been linked to inflammatory brain disorders, and their functional roles are still under investigation. Notably, within this group IL-8 shows the highest basal expression in NAHAs-conditioned media as determined by the analysis of the antibody array, whereas IL-1β, IL-3, and IL-16 are nearly undetectable in untreated NAHAs. It is known that upon Aβ and/or IL-1β stimulation, astrocytes release IL-8 [4], a critical mediator for neutrophils-endothelial cells and astrocytes-endothelial cells interactions in CNS inflammatory reactions [5].

IL-1β is a relevant cofactor in AD development risk [6,7], AD neuroinflammation [8-10], and acts as a co-promoter of ICAM-1/sICAM-1 [11-16] and of RANTES [17,18] expression in NAHAs.

In experimental co-culture models, IL-3 released from astrocytes supports the growth of microglia cells, inducing them to remove Aβ fibrils, and exerting in such way a neuroprotective role [19].

IL-16 is a chemokine inducing migration across the BBB and proliferation of $CD4^+$ immune cells and promoting the expression of other proinflammatory cytokines including IL-1β and IL-6. In traumatic CNS injury astrocytes, microglia, and also neurons release this cytokine. IL-16 levels are found to be increased in AD patients as an expression of immune activation in response to the presence of Aβ deposits [20].

Moreover, the constitutive secretion of two other agents also involved in neuroinflammation, i.e. chemokine MCP-1 and metalloproteinase inhibitor TIMP-2, was unaffected in cultured Aβ ± NPS 2143-treated NAHAs [Table 1; Figure S1]. Hence, it is feasible that Aβ-treated microglia might be the main source of MCP-1 surpluses in AD brains [9]. TIMP-2 is amply expressed in adult CNS and its level is increased in CSF of AD patients, as regulator of metalloproteinases activity plays an anti-inflammatory and neuroprotective role [21].

In addition, our results show, rather unexpectedly, that 96-h after the experiments onset, a delayed stimulation of PDGF-BB secretion over both control and fAβ alone-exposed astrocytes took place in NAHAs co-treated with fAβ+NPS 2143 [Table 1, Supp Fig. 2]. PDGF-BB is thought to be an important angiogenic promoter in the tissue replacement phase after CNS injury and a neuroprotective agent, especially in cognitive dysfunction [22].

Clearly, more studies are needed to unravel the detailed mechanisms regulating the expression of these agents in NAHAs and to clarify their roles in human AD's neuroinflammation.

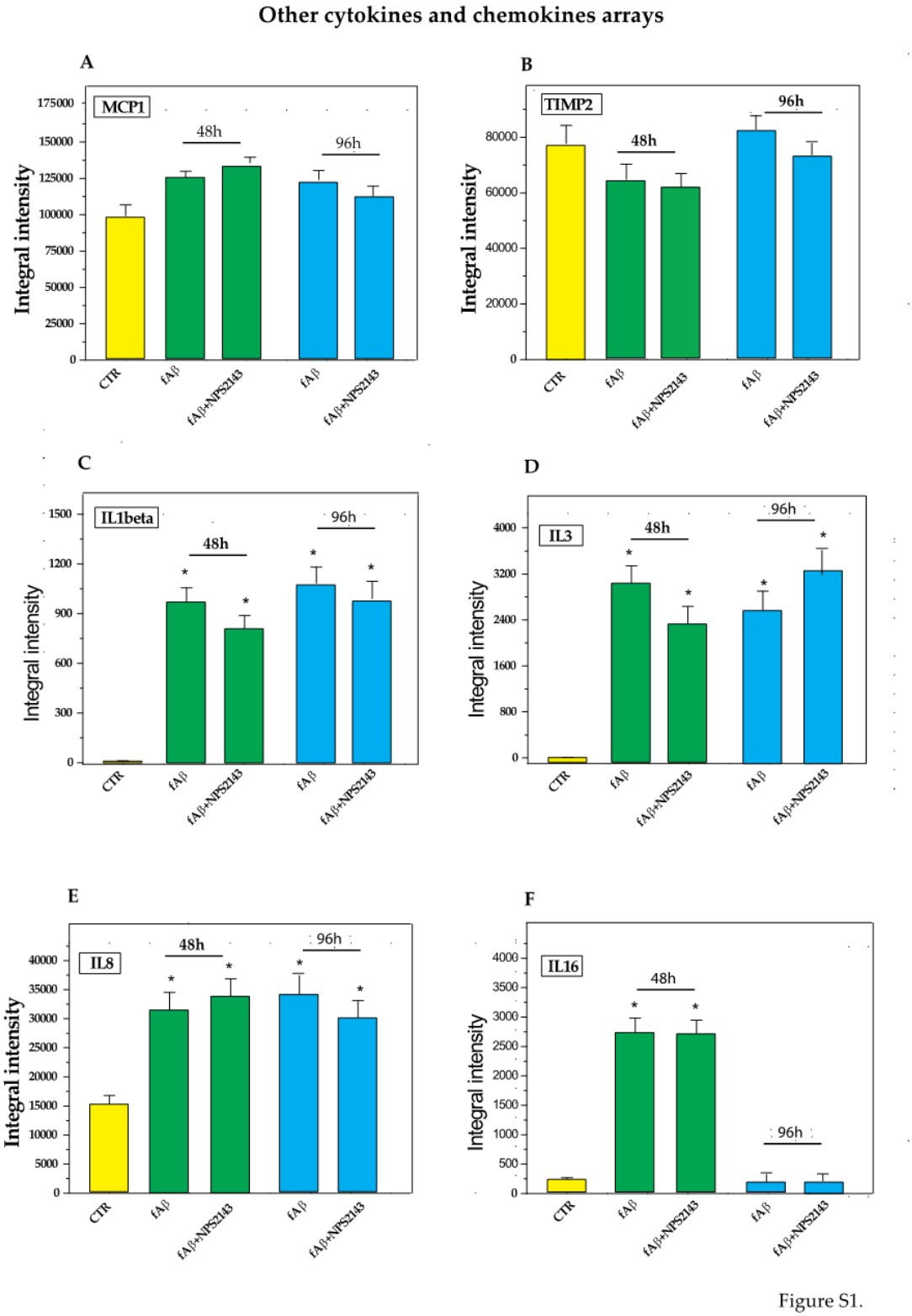

Figure S1.

Figure S1. Time-dependent expression of cytokines and chemokines (**A–F**) secreted by control and fAβ-treated NAHAs, the levels of which were unaffected by a co-treatment of fAβ with CaSR NAM NPS 2143. Each cytokine/chemokine was detected in the NAHA-conditioned media via membrane-based antibody array. The antibody array was analyzed with an Odissey™ (LI-COR) scanner and the positive staining intensities were quantified using the Image Studio™ (v.5.2) software. The integral intensities of the positive signals from each array were normalized via comparisons to corresponding positive controls. Results were expressed as mean values ± SEM. *P* values were calculated via one-way ANOVA followed by post hoc Tukey's test. * $P < 0.05$ *vs*. controls (CTR). fAβ, $fA\beta_{25-35}$.

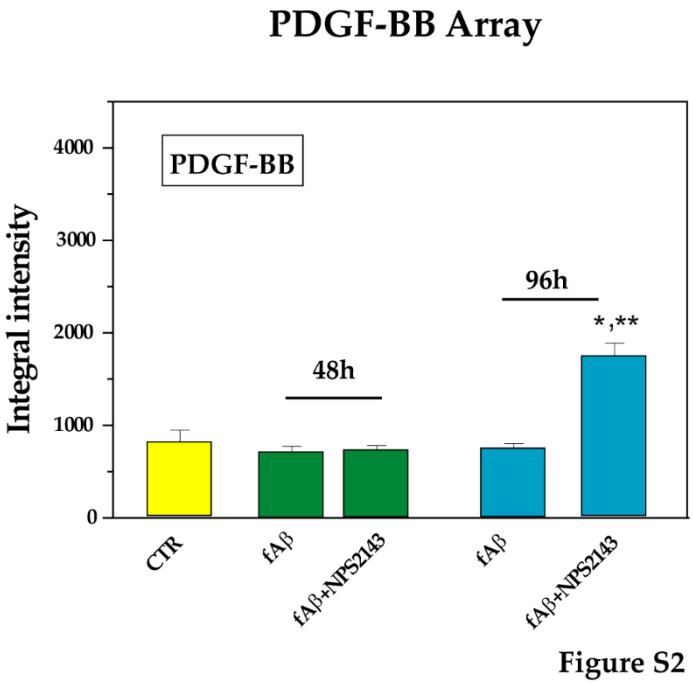

Figure S2. The secreted PDGF-BB levels pattern behaves differently from the all the other cytokines and chemokines we tested in the NAHA-conditioned media via membrane-based antibody array, because its basal levels did not change with respect to controls in fAβ-treated NAHAs for up to 96 h, but did increase at 96 h in fAβ+NPS 2143 co-treated NAHAs. The antibody array was analyzed with an Odissey™ (LI-COR) scanner and the positive staining intensities of PDGF-BB were quantified using the Image Studio™ (v.5.2) software. fAβ, $fA\beta_{25-35}$. The integral intensities of the positive signals from each array were normalized via comparisons to corresponding positive controls. Results were expressed as mean values ± SEM. *P* values were calculated via one-way ANOVA followed by post hoc Tukey's test. * $P < 0.05$ vs. controls (CTR); ** $P < 0.05$ vs. fAβ. fAβ, $fA\beta_{25-35}$.

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