

Extracellular vesicle-mediated amyloid transfer to neural progenitor cells: implications for RAGE and HIV infection

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Supplemental Figures

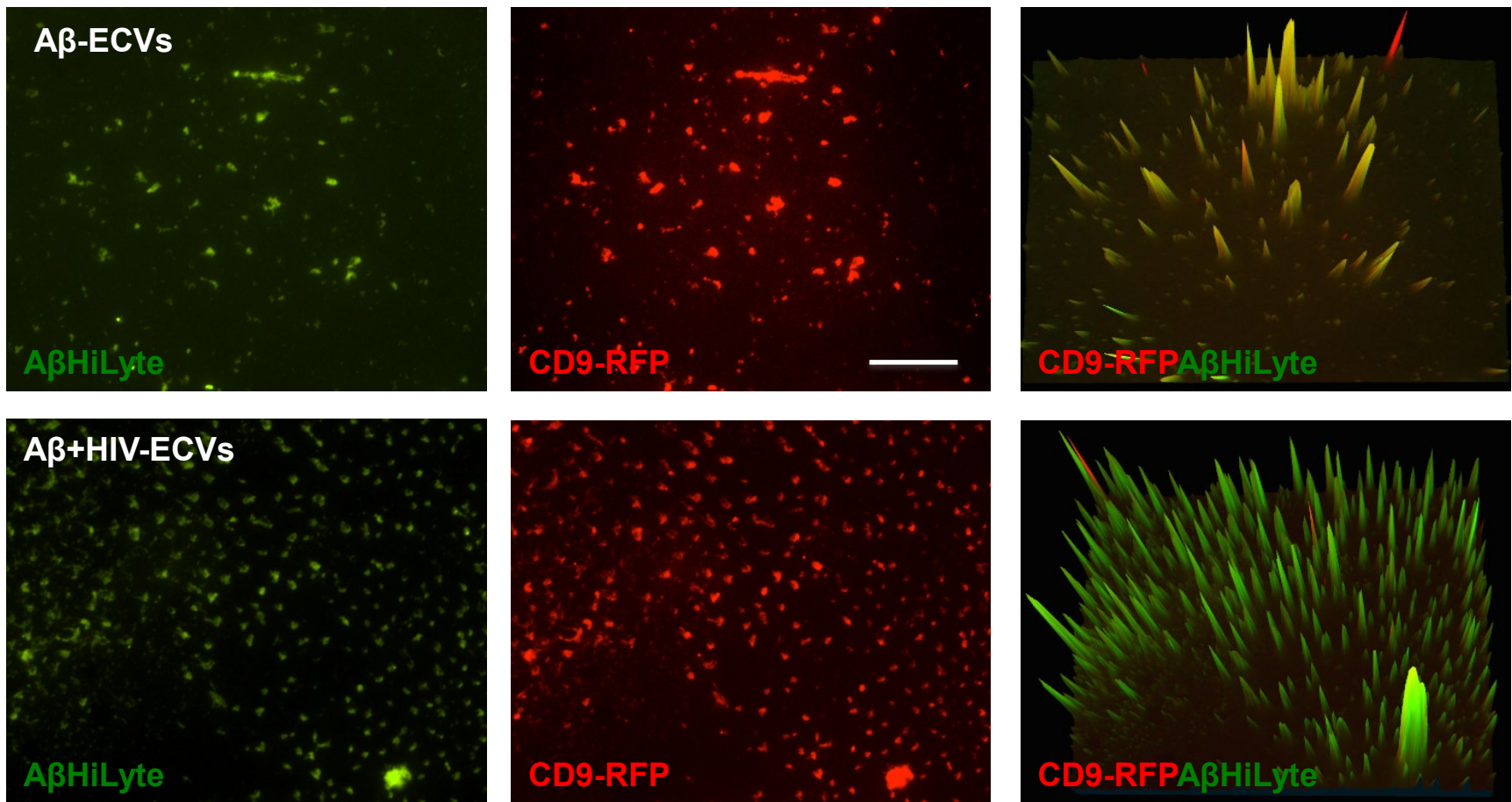
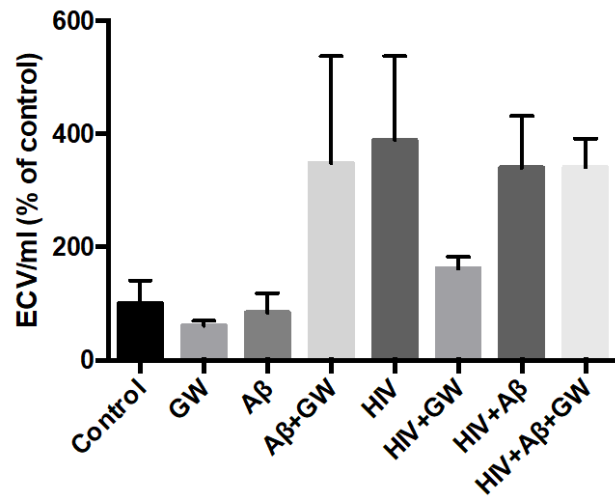
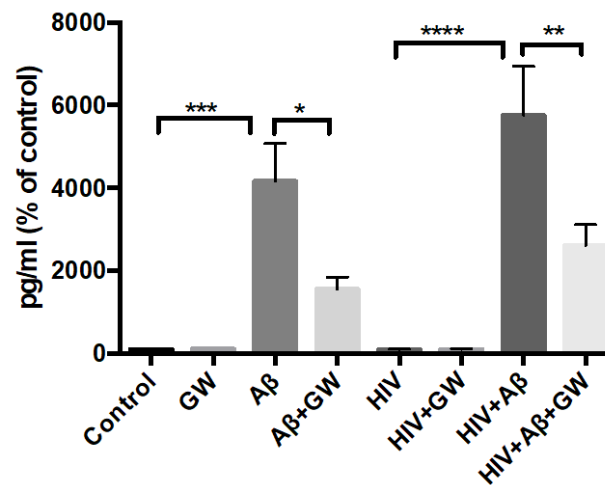
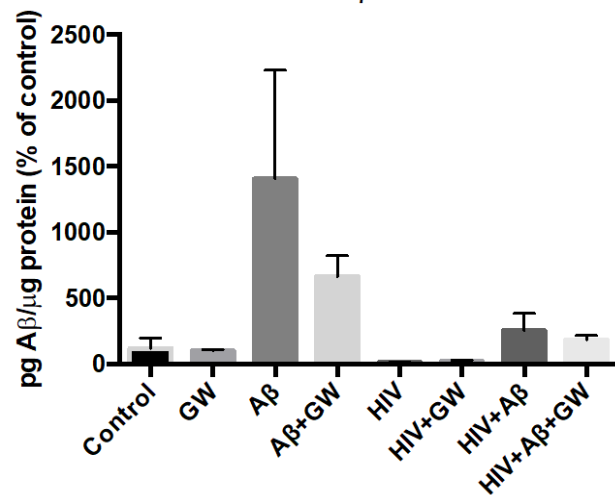
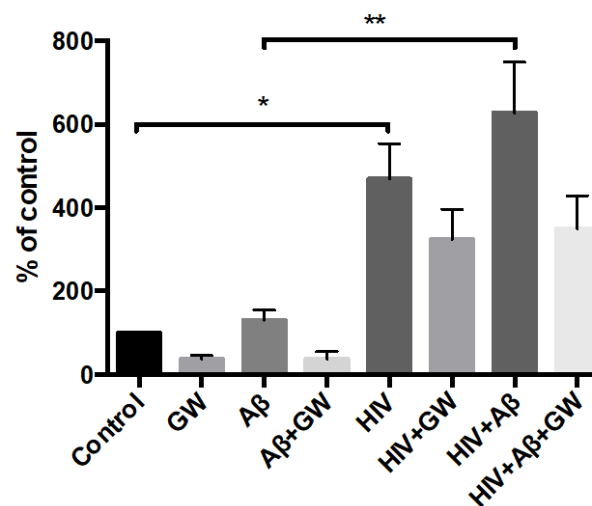


Figure S1. HBMEC-derived ECVs. HBMEC were transfected with the CD9 Cyto-Tracer construct pT CD9-RFP (System Biosciences) using Purefection Transfection Reagent following the manufacturer's protocol. Twenty four hours post transfection, cells were exposed to HIV (30 ng/ml) and/or 100 nM A β (1–40) HiLyte AlexaFluor-488 (green) for 48 h as described previously (Andras et al., Mol Cell Neurosci 79:12-22, 2017). Green and red fluorescent ECV were isolated from the culture media and imaged by fluorescence microscopy (scale bar: 20 μ m). The right panels show the intensity surface plots for the merged images. Note that the green fluorescent A β is associated with ECVs of different sizes. Most of the CD9-RFP positive ECVs have fluorescent A β cargo.

A**Total ECV number****B****ECV A β levels****C****ECV A β levels****D****ECV protein levels**

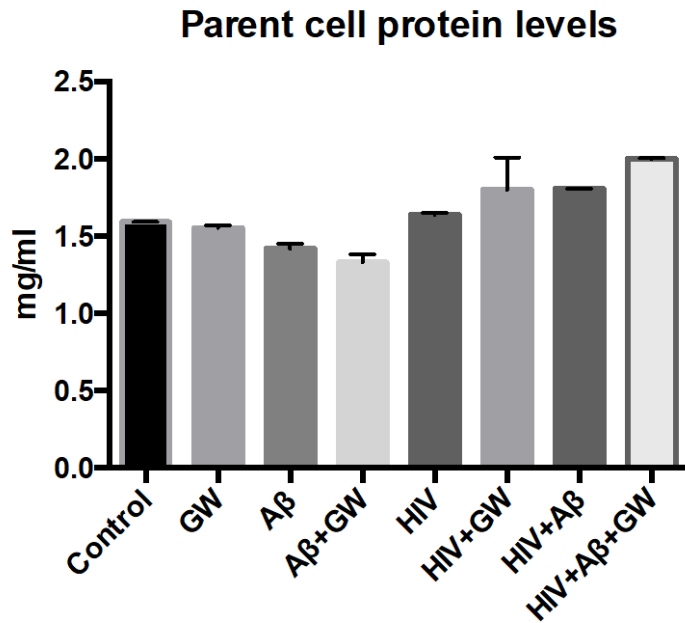
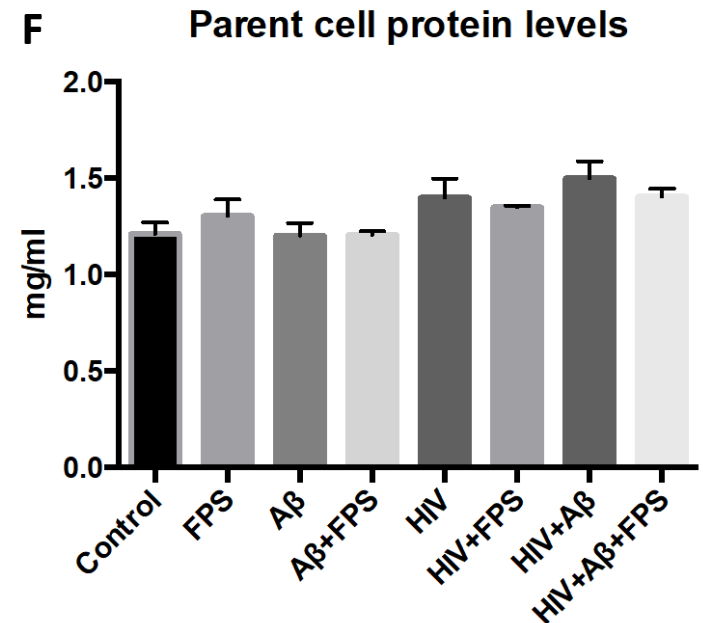
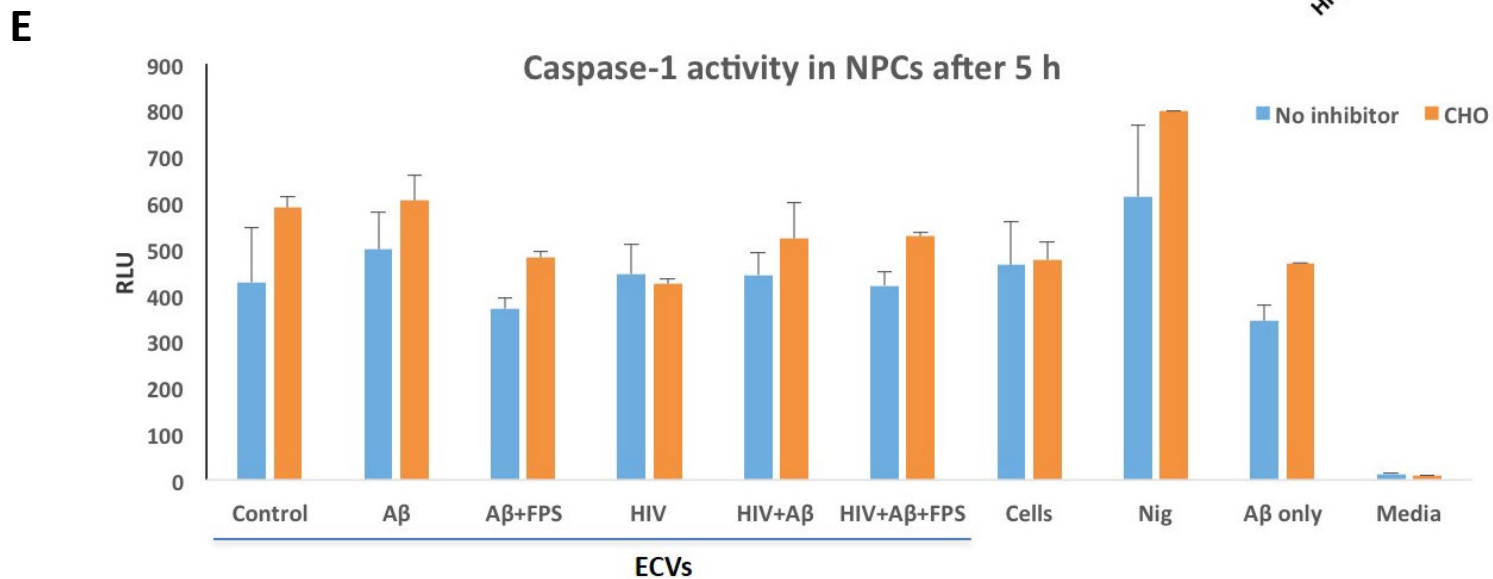
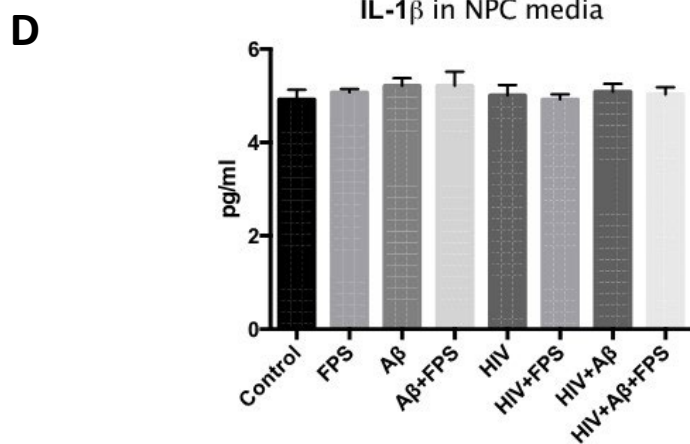
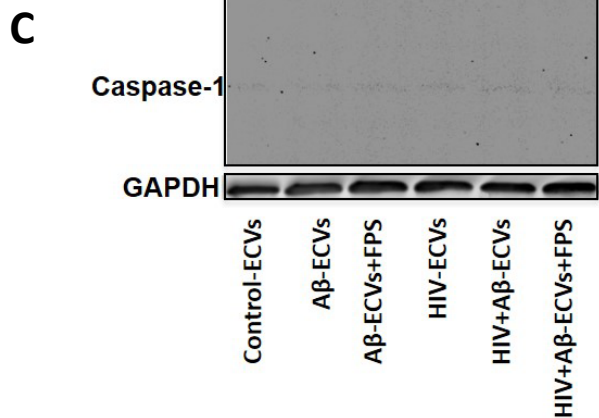
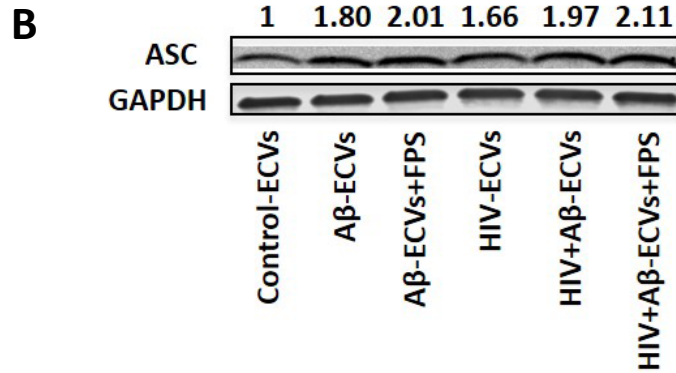
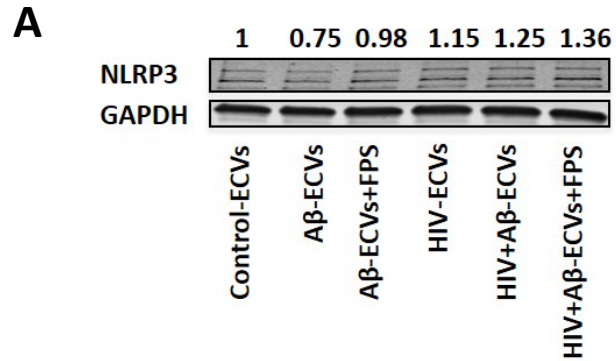
E**F**

Figure S2. The effects of nSMase inhibition on brain endothelial ECV release and ECV-A β levels. HBMEC were exposed to 30 ng/ml HIV particles and/or 100 nM A β (1-40) for 48 h. Selected cultures were pretreated with 20 μ M GW4869 for 1 h followed by cotreatment with 30 ng/ml HIV particles and/or 100 nM A β (1-40) for 48 h. ECVs were isolated from the culture media. **A)** Total ECV number as measured by nanoparticle tracking analysis (NTA). Values are mean \pm SEM, n=3-4. **B & C)** Total ECV A β (1-40) levels as measured by ELISA and normalized either to **B)** media volume or **C)** ECV protein levels. Values are mean \pm SEM, n=7. **D)** ECV protein levels as measured by BCA assay. **E&F)** Parent cell protein levels as measured by BCA assay. Values are mean \pm SEM, n=3. Statistically significant at *p<0.05, **p<0.01, ***p<0.001, or ****p<0.0001.



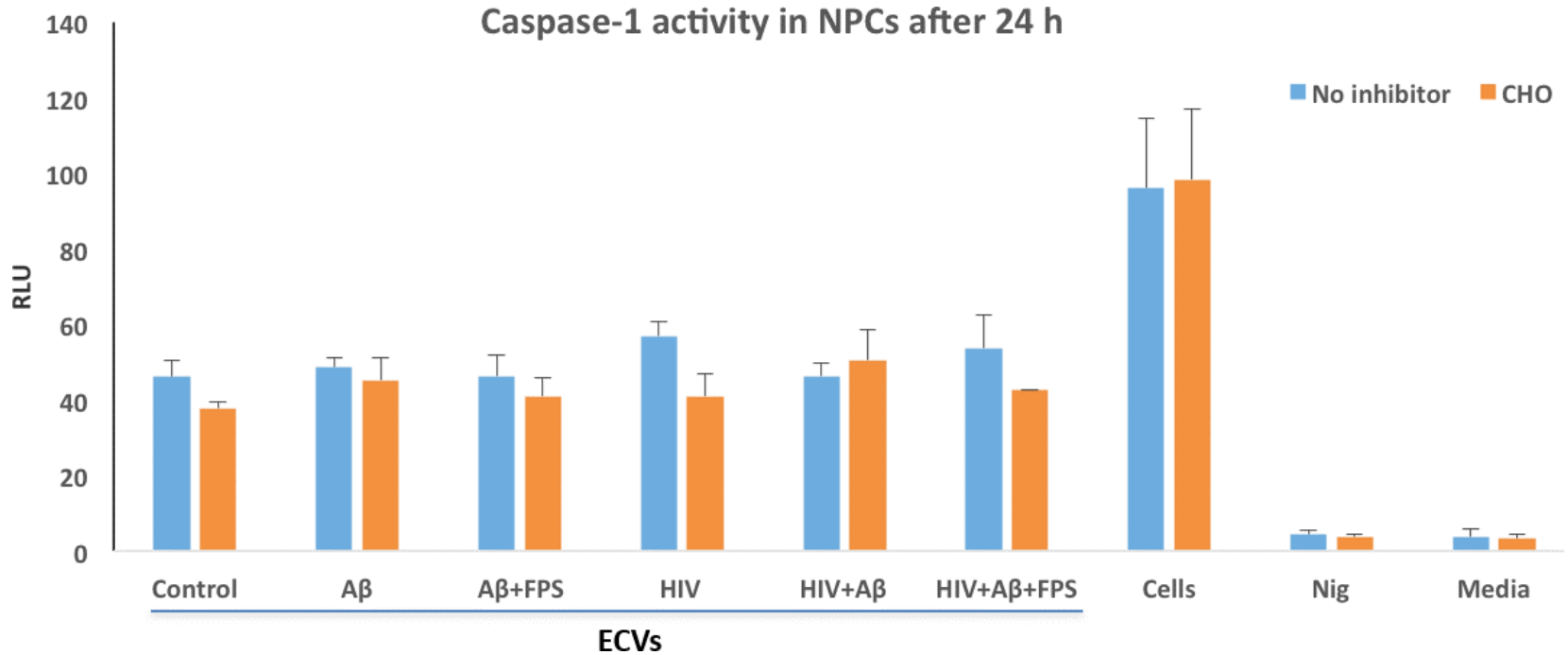
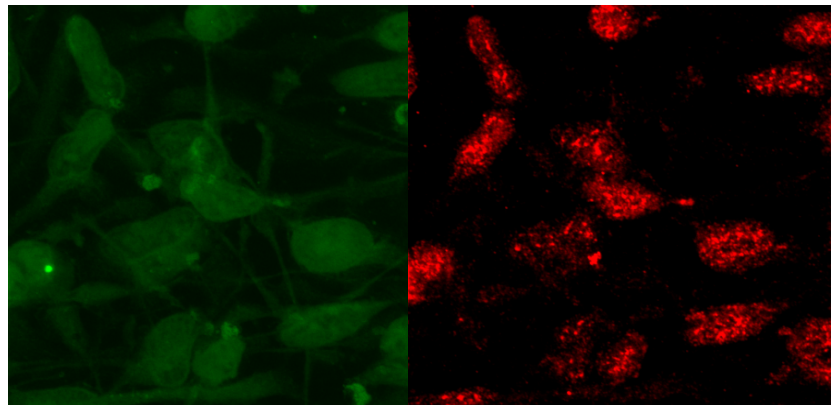
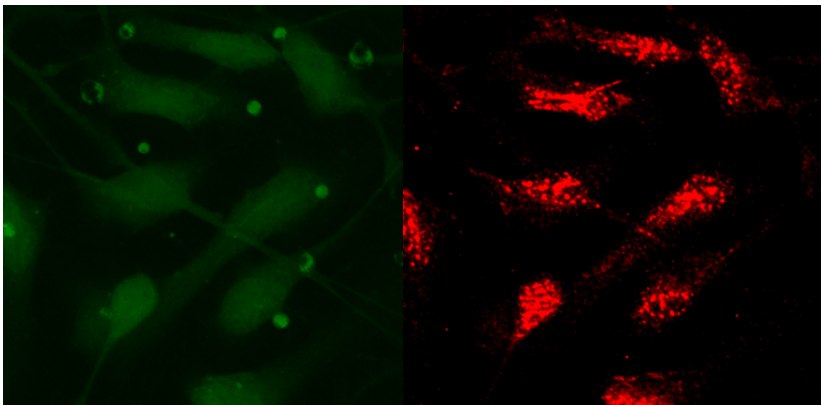
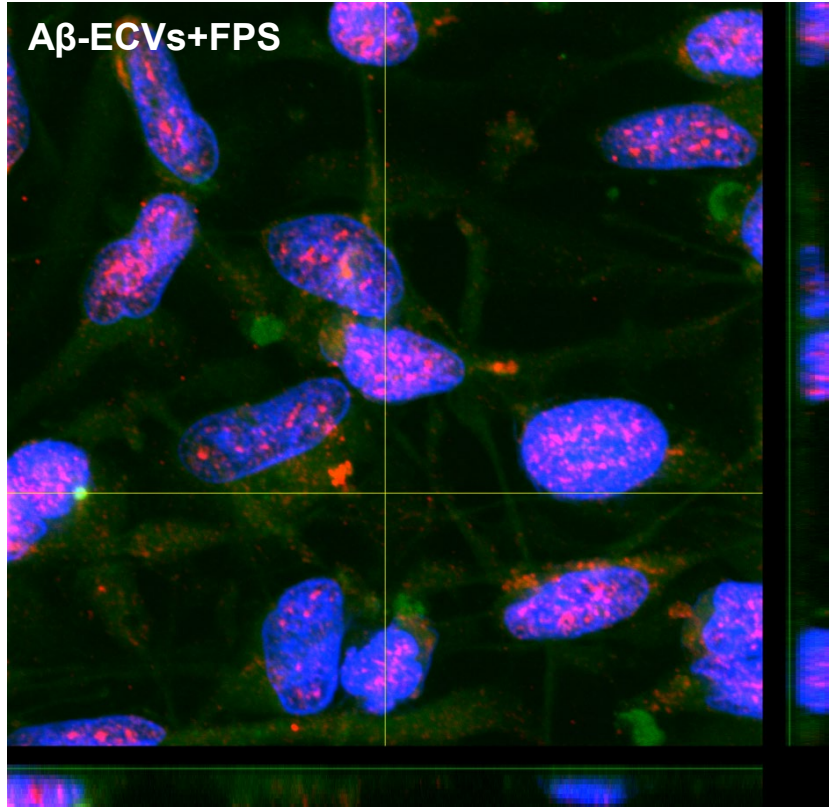
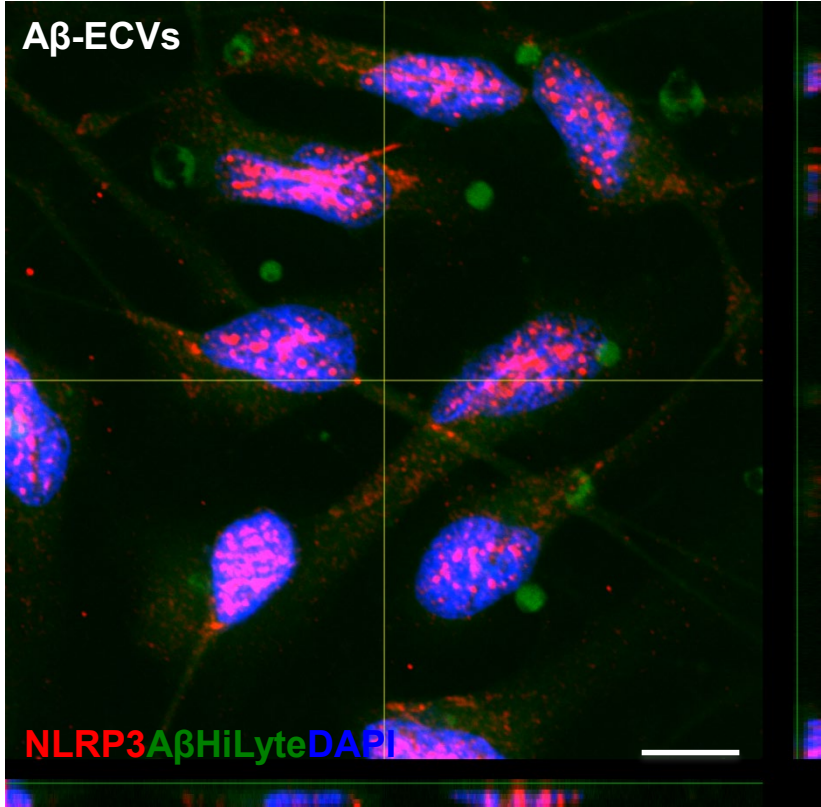
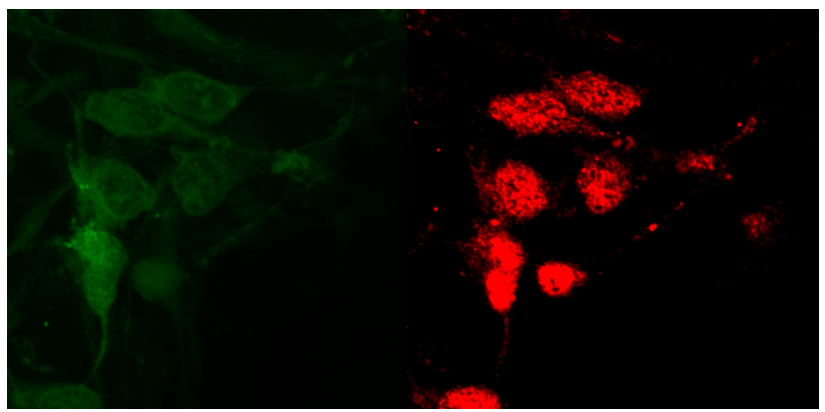
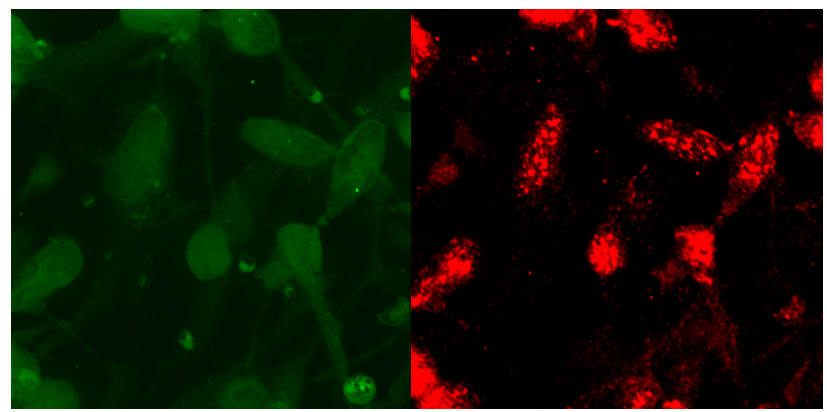
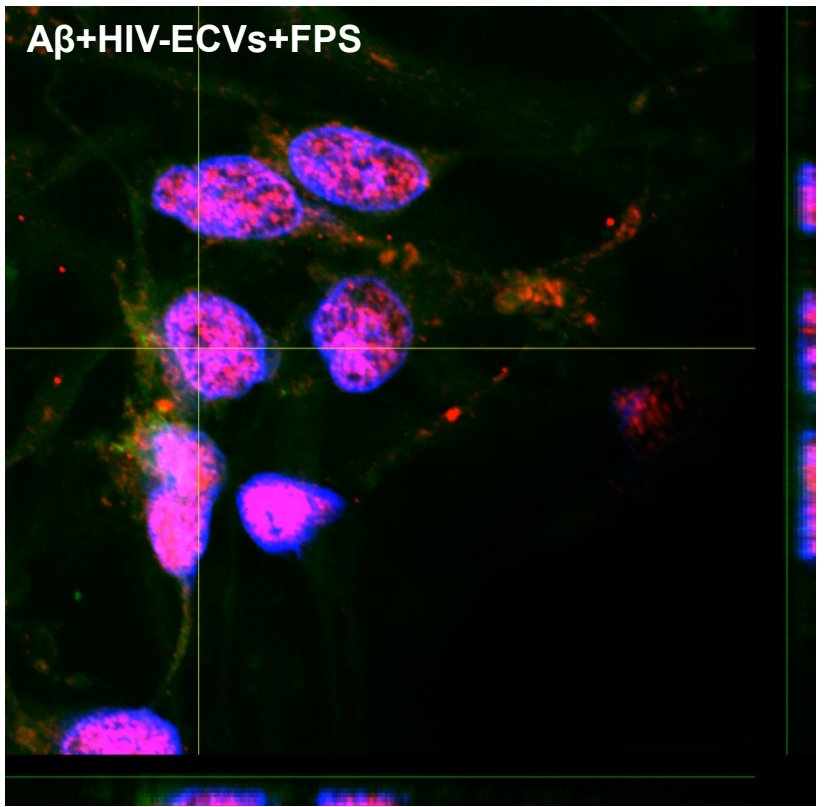
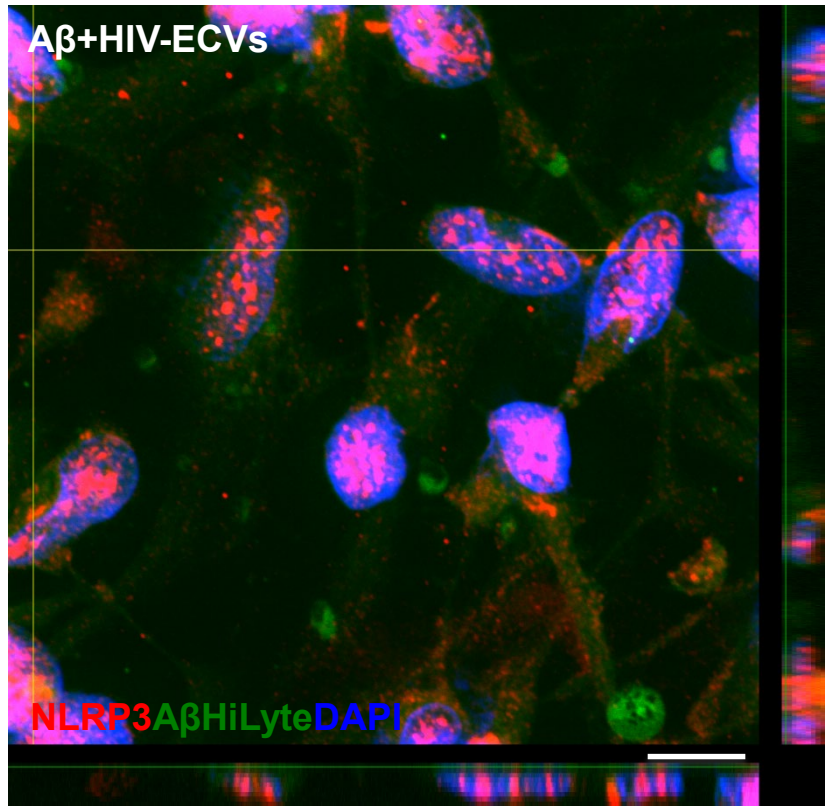


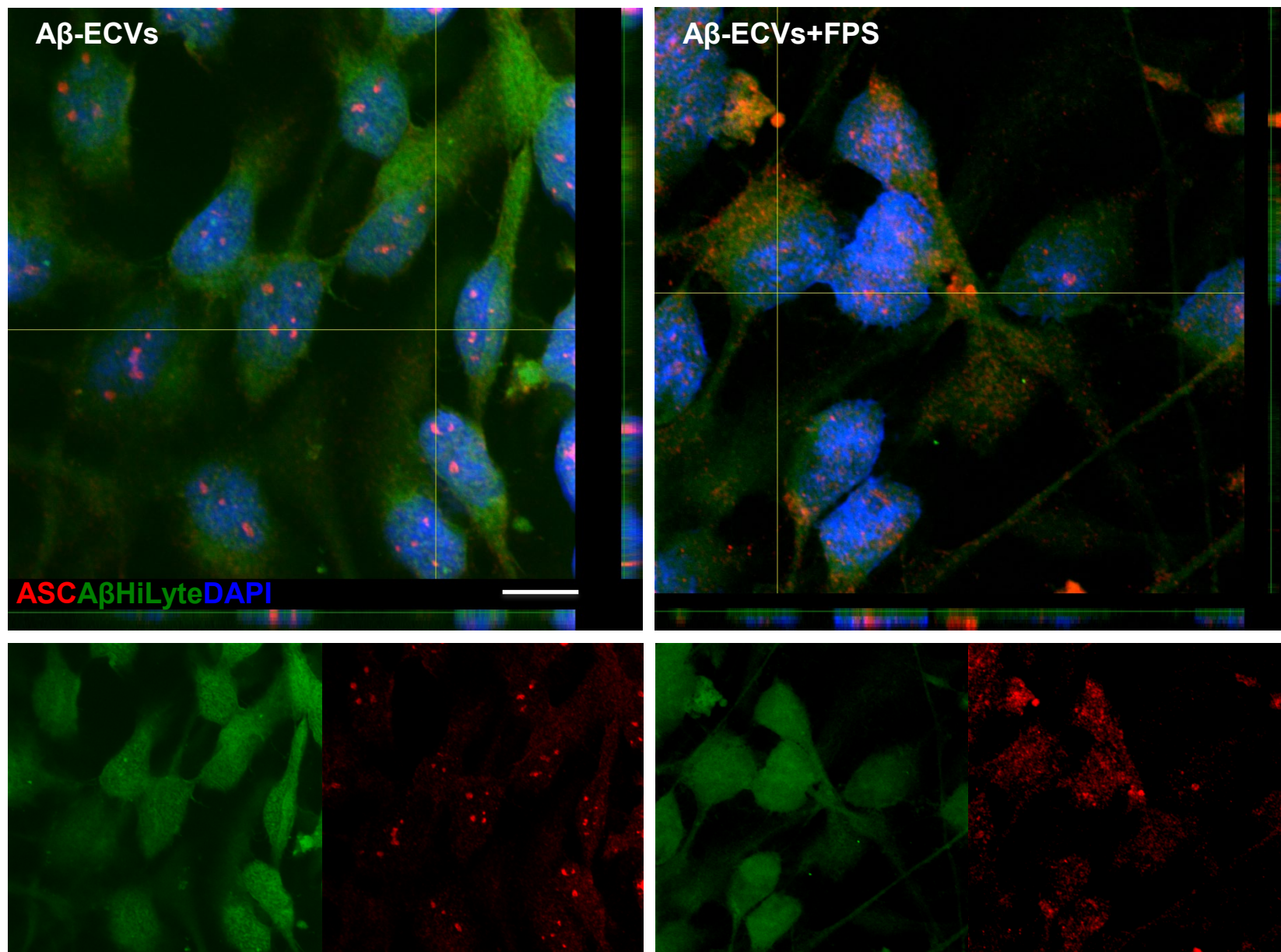
Figure S3. Analysis of NLRP3, ASC, caspase-1, and IL-1 β . HBMEC were treated with HIV and/or A β and ECVs were isolated as in Figure S2. Then, human NPCs were exposed to HBMEC-derived NPCs for 24 h, with selected cultures pretreated with 500 nM FPS-ZM1 (FPS) for 2 h followed by cotreatment with the isolated ECVs. Immunoblotting for **A)** NLRP3, **B)** ASC and **C)** caspase-1. GAPDH was assessed as a loading control. **D)** IL-1 β in the NPC culture media was undetectable 24 h after ECV exposure. IL-1 β levels were detected once by ELISA 3 days after differentiation as shown on the graph. **E)** NPC caspase-1 activity was measured 5 h and 24 h after ECV exposure by the Caspase-Glo 1 inflammasome assay with or without the specific caspase-1 inhibitor YVAD-CHO (CHO). Cells without ECVs treatment were used as additional control. Nigericin (Nig, 20 μ M) and A β (1 μ M) were used as positive controls. Cell culture media alone was used as a negative control. Luminescence was recorded after 60 minutes. Values are mean \pm SEM, n=4 (no inhibitor) and n=2 (CHO).

A



A



B

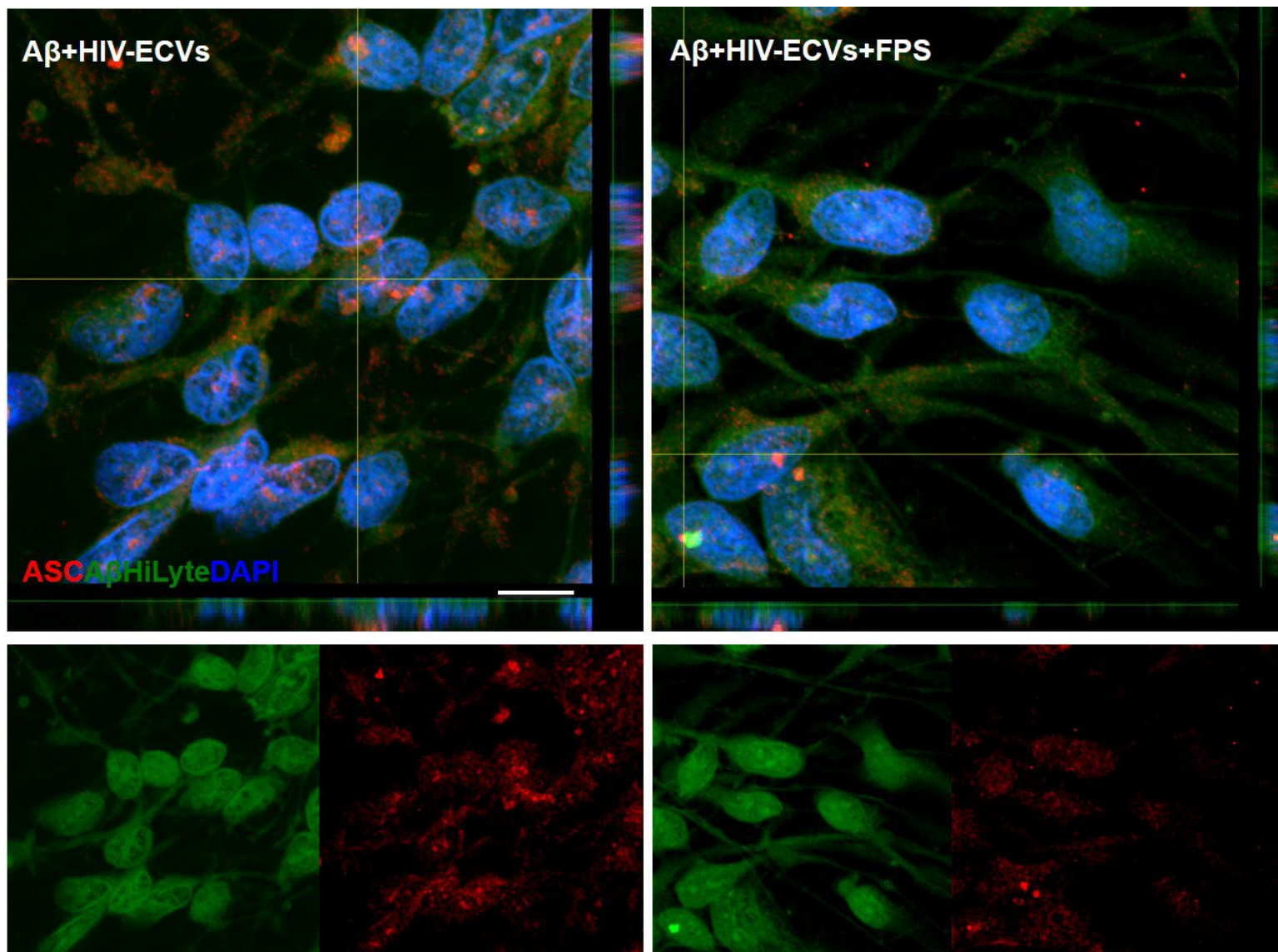
B

Figure S4. Impact of HBMEC-derived ECVs on colocalization of NLRP3 and ASC with A β in NPCs. Related to **Figure 4**. HBMEC were treated with HIV and/or A β and ECVs were isolated as in **Figure S2**; however, A β (1-40) HiLyte was used instead of non-fluorescent A β (1-40). Then, human NPCs were exposed to HBMEC-derived ECVs for 24 h, with selected cultures additionally treated with 500 nM FPS-ZM1 (FPS) as in **Figure S3**. A) NLRP3 and B) ASC immunoreactivity is shown in red. DAPI staining (blue) visualizes the NPC nuclei. Transferred A β (1-40) HiLyte from brain endothelial ECVs is visualized in green. The combined z-stack images are representative from three experiments. Scale bar: 10 μ m.

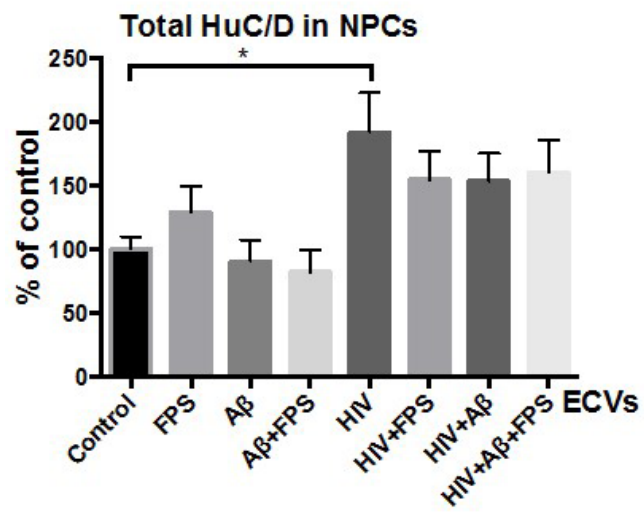
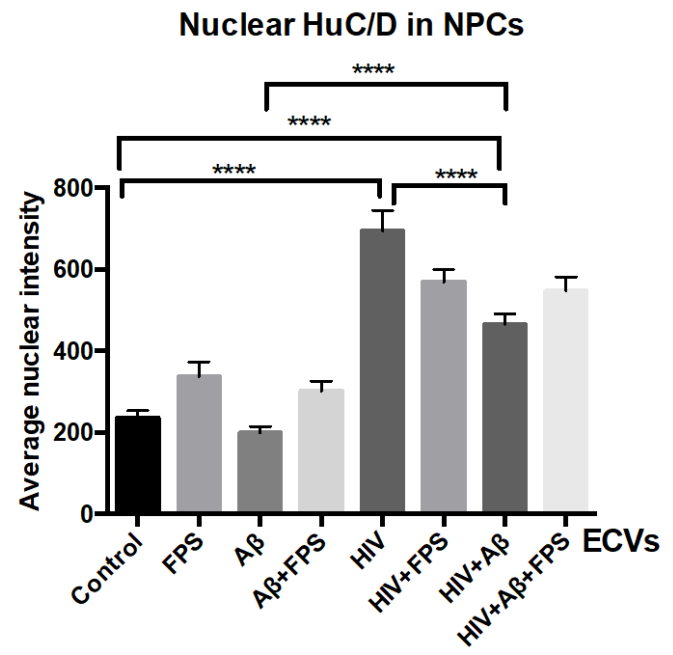
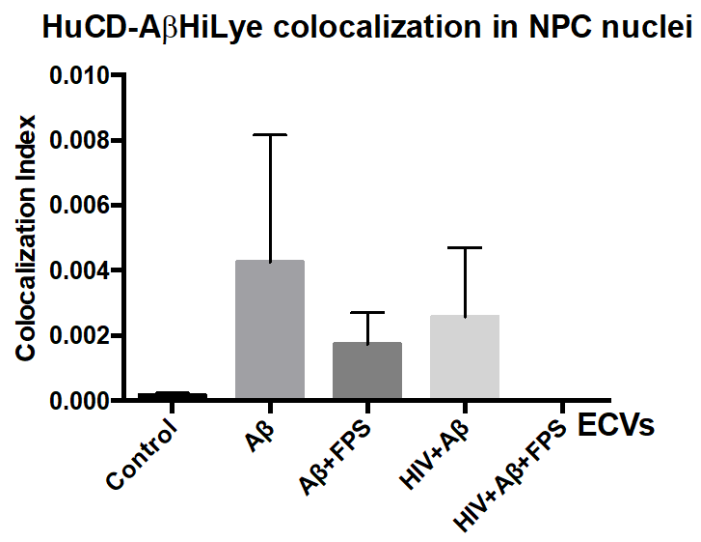
A**B****C**

Figure S5. Impact of ECV-mediated A β transfer on NPC differentiation as measured by HuC/D. HBMEC were exposed to HIV (30 ng/ml) and/or 100 nM A β (1-40) HiLyte for 48 h, followed by isolation of ECVs from the cell culture media. NPC were differentiated for 3 days in the presence of HBMEC-derived ECVs. At the beginning of differentiation, selected NPC cultures were pretreated with 500 nM FPS-ZM1 (FPS) for 2 h followed by cotreatment with the isolated ECVs. At the end of the 3-day differentiation, the neuronal marker Hu C/D was assessed by confocal microscopy. A) Total intensity of Hu C/D immunoreactivity as quantified from the confocal images. B) Intensity of nuclear Hu C/D immunoreactivity as quantified from random nuclear areas. C) Quantification of Hu C/D colocalization with A β (1-40) HiLyte in random nuclear areas. Values are mean \pm SEM, n=28 (total); n=179-180 (nuclear); n=120 (colocalization). Statistically significant at *p<0.05, ****p<0.0001.

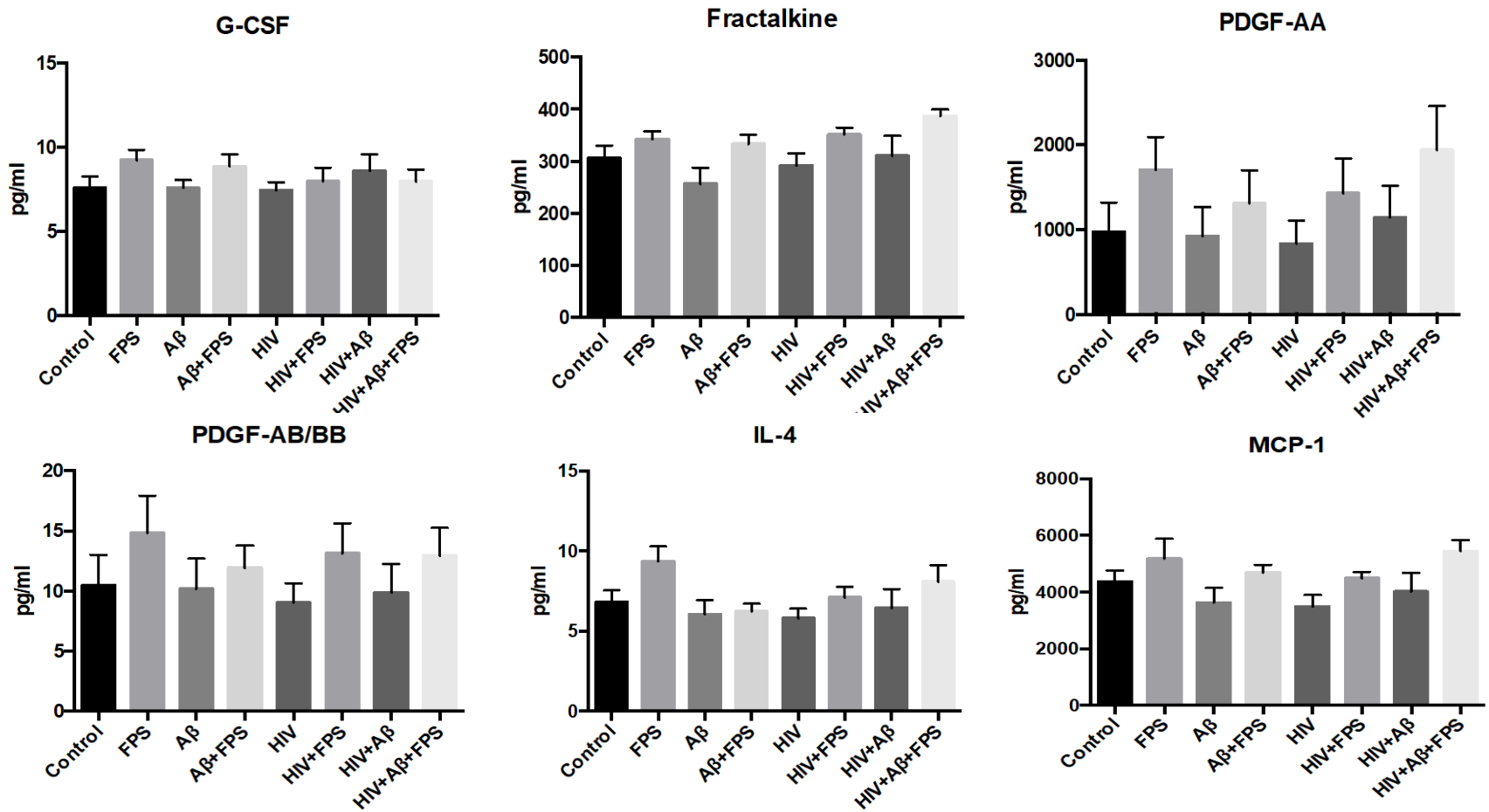


Figure S6. Cytokine/chemokine panel after NPC differentiation. NPC were treated and differentiated as in **Figure 5**. At the end of the 3-day differentiation, G-CSF, IL-4, MCP-1, Fractalkine, PDGF-AA, PDGF-AB/BB levels were measured by Luminex MagPix assay from the cell culture media. Values are mean \pm SEM, n=5-8. NS, not statistically significant