

Neural stem cell extracellular vesicles restore endotoxin-induced dysfunction in PSEN2 human iPSC-derived brain endothelial cells

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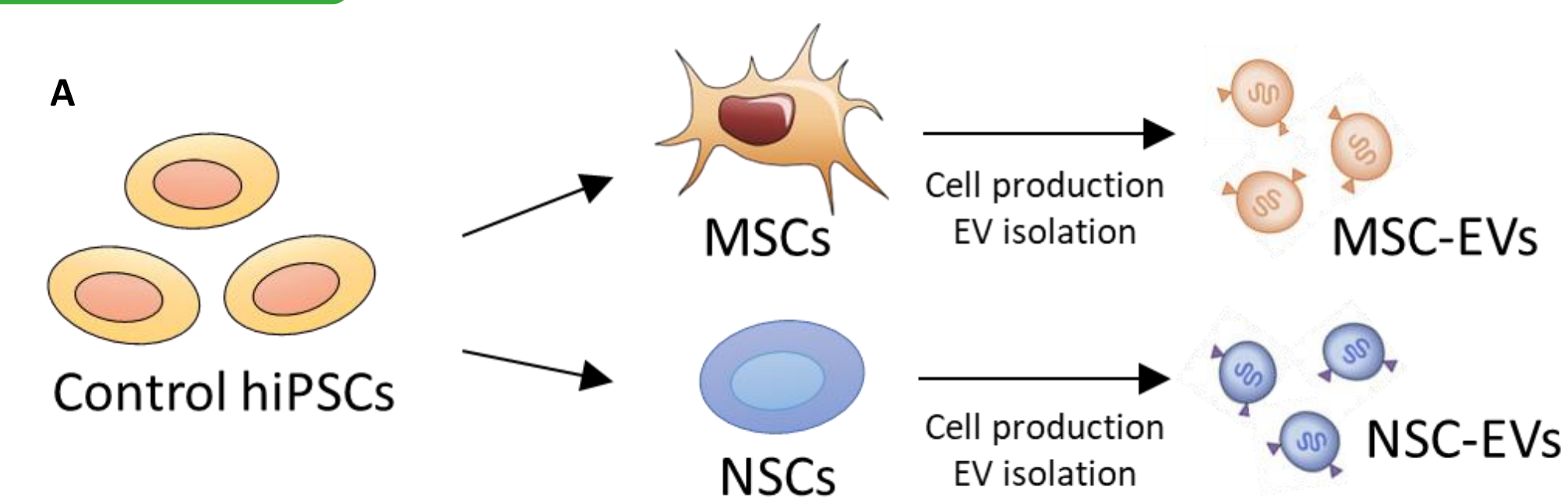
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Introduction

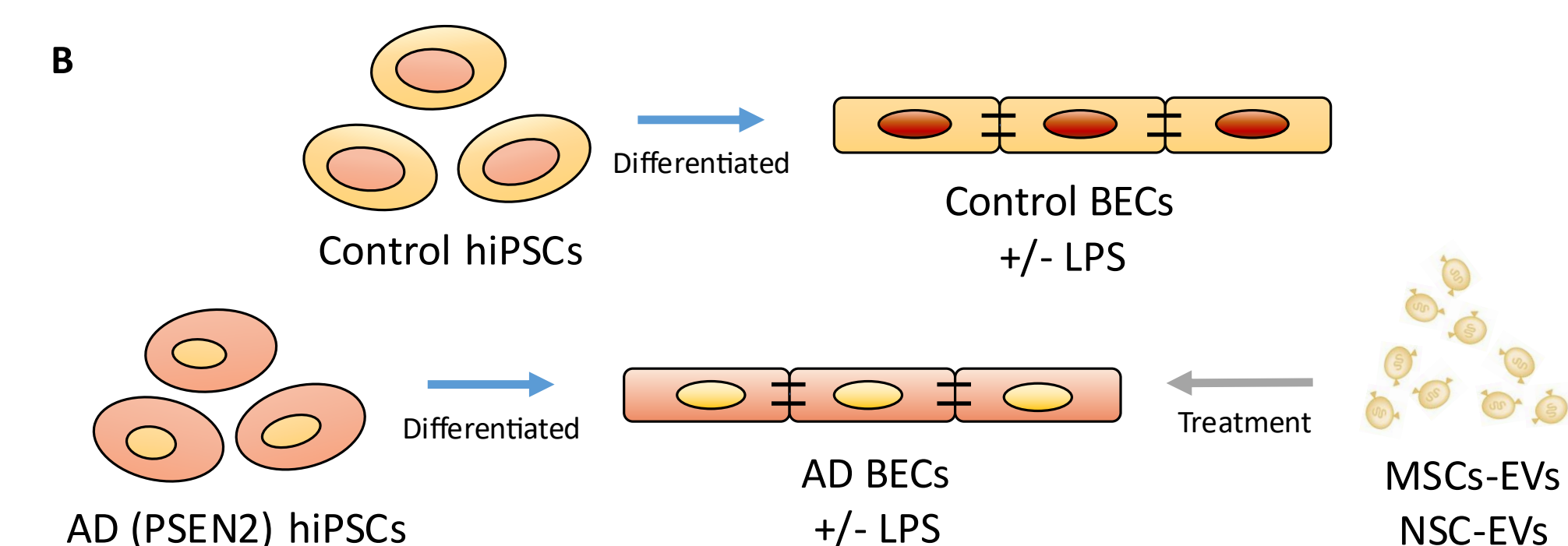
- In Alzheimer's disease (AD), cells of the blood-brain barrier (BBB), brain endothelial cells (BECs), exhibit impaired barrier properties [1]
- Stem cell-derived extracellular vesicles (EVs) have been found to reduce neurodegeneration and cognitive deficits in AD, possibly in part by restoring BBB function [1]
- It is unclear what cell source is the most promising for clinical translation of EVs as therapeutics in AD

The aims of this study were to i) compare the yield and composition of EVs from neural stem cells (NSCs) and mesenchymal stem cells (MSCs) from the same human induced pluripotent stem cell (hiPSC) line, and ii) compare their function in inhibiting endotoxin-induced dysfunction in hiPSC-derived BECs harbouring an AD PSEN2 mutation.

Methods



A) Healthy control hiPSCs were differentiated to MSCs using a STEMdiff Mesenchymal Progenitor Kit and to NSCs using a STEMdiffTM SMADi Neural Induction Kit (STEMCELL Technologies). MSCs were characterized by flow cytometry and tri-lineage differentiation to cartilage, bone, and fat. NSCs were characterized by positive expression of NSC markers Pax6, Nestin, and Sox2 using immunocytochemistry (ICC). Small EVs were isolated from both MSCs and NSCs using differential ultracentrifugation and characterized by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and quantification of neurotrophic and immunomodulatory proteins and EV markers (CD63, TSG101).



B) Functional testing of EVs was done in an AD cell model: hiPSCs expressing a familial AD PSEN2 mutation differentiated to BECs [2] and challenged with lipopolysaccharide (LPS) to induce an inflammatory-like response. EVs were added at the same time as LPS, and effects were evaluated after 24 hours. BECs were fixed and stained for tight junction markers Occludin and Claudin-5. Cells were imaged using a BioTek Cytation 5 and mean fluorescent intensity (MFI) was quantified using ImageJ. Medium was collected to quantify lactate dehydrogenase (LDH) and inflammatory cytokine concentrations. MTT and DCFDA assays were used to measure metabolic activity as an estimate of viability and proliferation, and reactive oxygen species (ROS), respectively.

References

- Phelps *et al.* Extracellular vesicles for the treatment of Alzheimer's disease: a systematic review. *Journal of Extracellular Biology*, 2025, 4(8):e70077.
- Neal *et al.* A simplified, fully defined differentiation scheme for producing blood-brain barrier endothelial cells from human iPSCs. *Stem Cell Reports*, 2019, 12(6):1380-1388.

Results

MSCs and NSCs were differentiated from healthy hiPSCs:

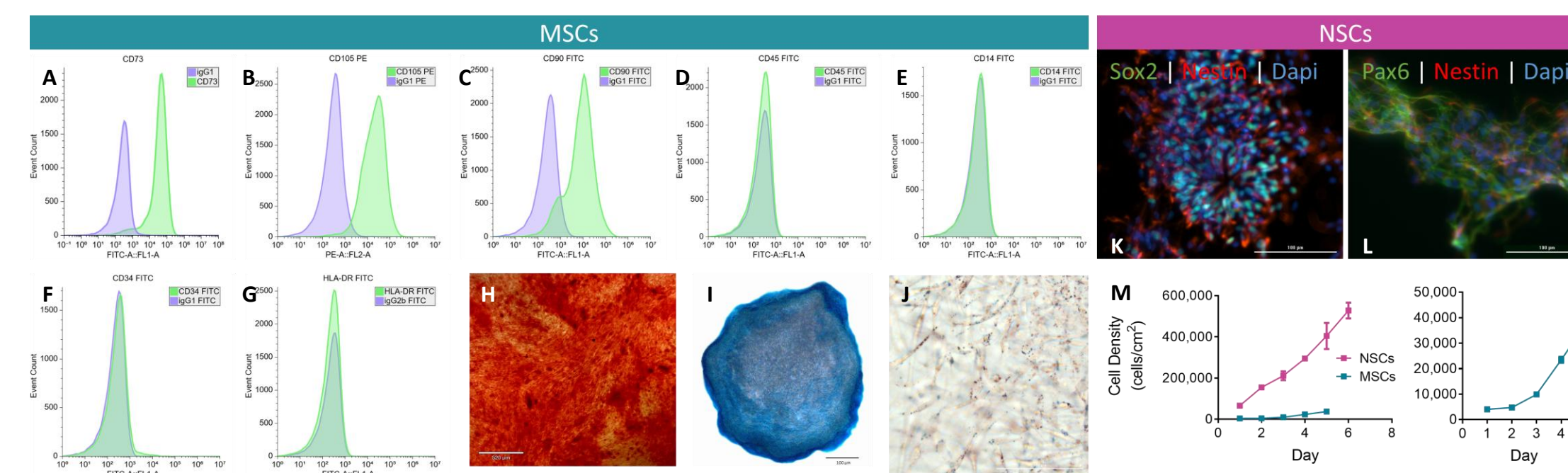


Figure 1. Characterization of MSCs and NSCs. Flow cytometry ensured MSC positive expression of CD73 (A), CD105 (B), and CD90 (C), and negative expression of CD45 (D), CD14 (E), CD34 (F), and HLA-DR (G). Tri-lineage differentiation confirmed MSCs' ability to differentiate to bone (H), cartilage (I), and fat (J) by staining with Alizarin red, Alcian blue, and Oil Red O respectively. ICC confirmed positive expression of Sox2, Nestin, and Pax6 for NSCs (K, L). Growth curves are shown for NSCs day 1-6 compared to MSCs day 1-5 (M).

MSCs produce a higher yield of EVs that contain more neurotrophic, survival, and immunomodulatory proteins compared to NSC-EVs:

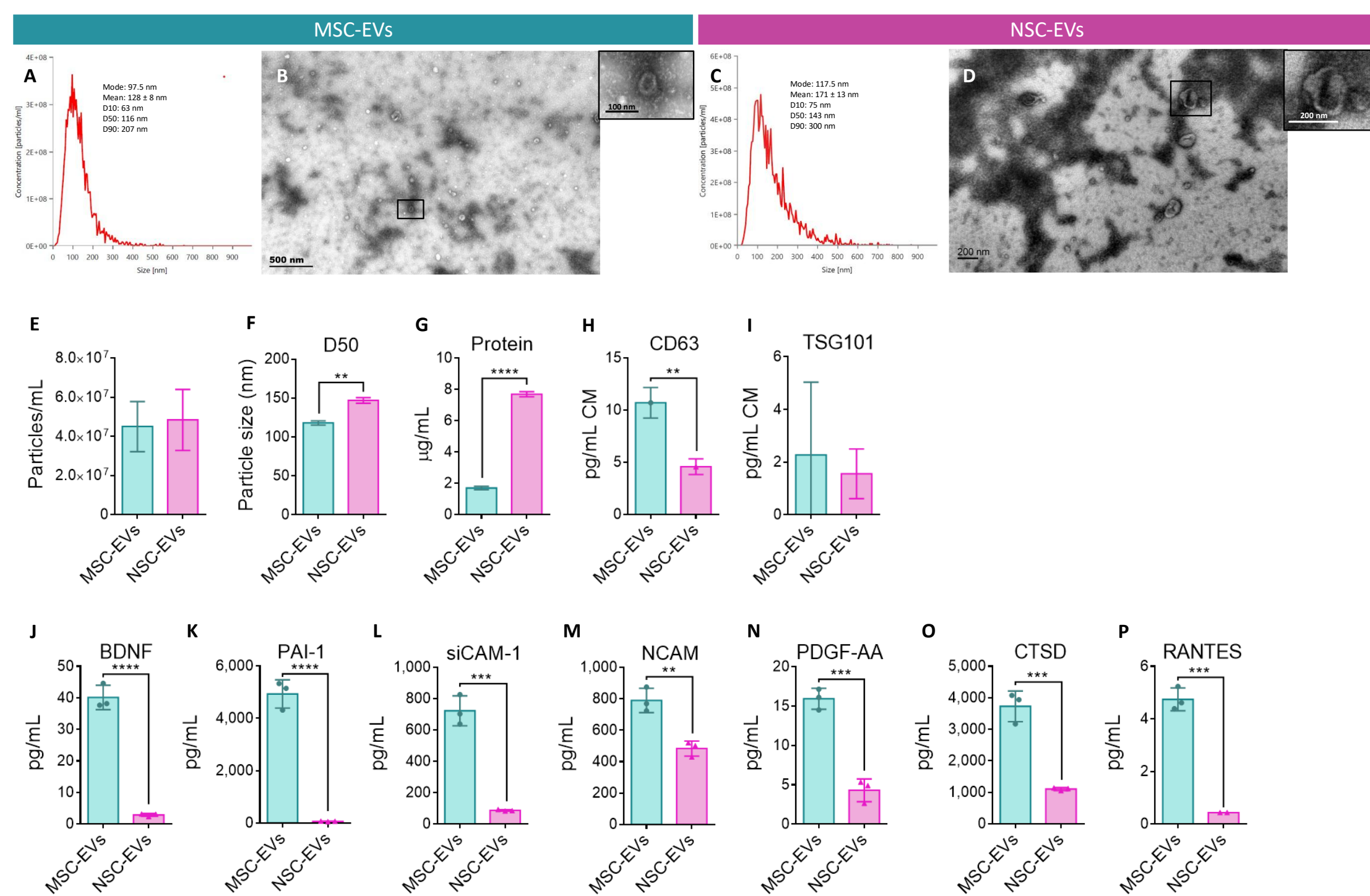


Figure 2. Characterization of isolated small MSC- and NSC-EVs, including concentration and size measured by NTA (A, C, E, F), morphology by TEM – wide view and zoomed in (B, D), total protein (G), concentration of EV markers CD63 (H) and TSG101 (I), and concentration of biomarkers in MSC- and NSC-EV fractions, including brain-derived neurotrophic factor (BDNF) (J), plasminogen activator inhibitor (PAI)-1 (K), soluble intercellular adhesion molecule (sICAM)-1 (L), neural cell adhesion molecule (NCAM) (M), platelet derived growth factor (PDGF)-AA (N), cathepsin D (CTSD) (O), and RANTES (P).

LPS induced inflammatory response in both healthy and AD BECs, partially restored by MSC-EVs:

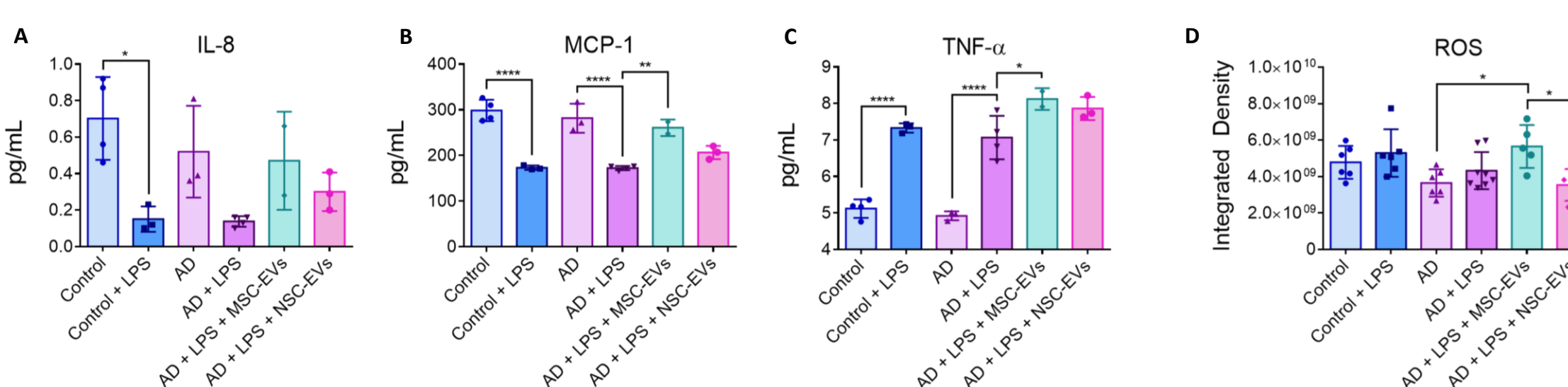


Figure 5. Cytokine concentrations measured in BEC cell medium after 24 hours of exposure to LPS and/or MSC- or NSC-EVs: IL-8 (A), MCP-1 (B), and TNF-α (C). Reactive oxygen species (ROS) measured by integrated density of DCFDA fluorescence (D).

BECs were differentiated from healthy and AD hiPSCs:

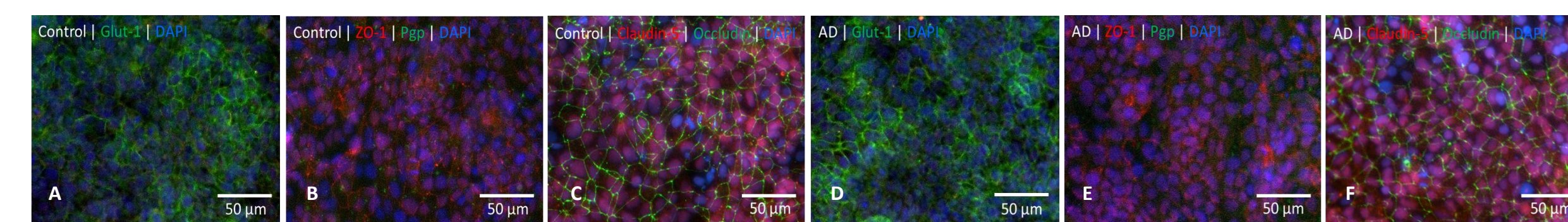


Figure 3. Characterization of control and AD (PSEN2) BECs. ICC for Glut-1 (A,D), ZO-1 (B, E), Pgp (B, E), Claudin-5 (C, F), and Occludin (C, F) confirmed successful differentiation to BECs.

LPS significantly reduced tight junction protein expression in AD but not control BECs, restored by NSC- and MSC-EVs, with NSC-EVs having a larger effect:

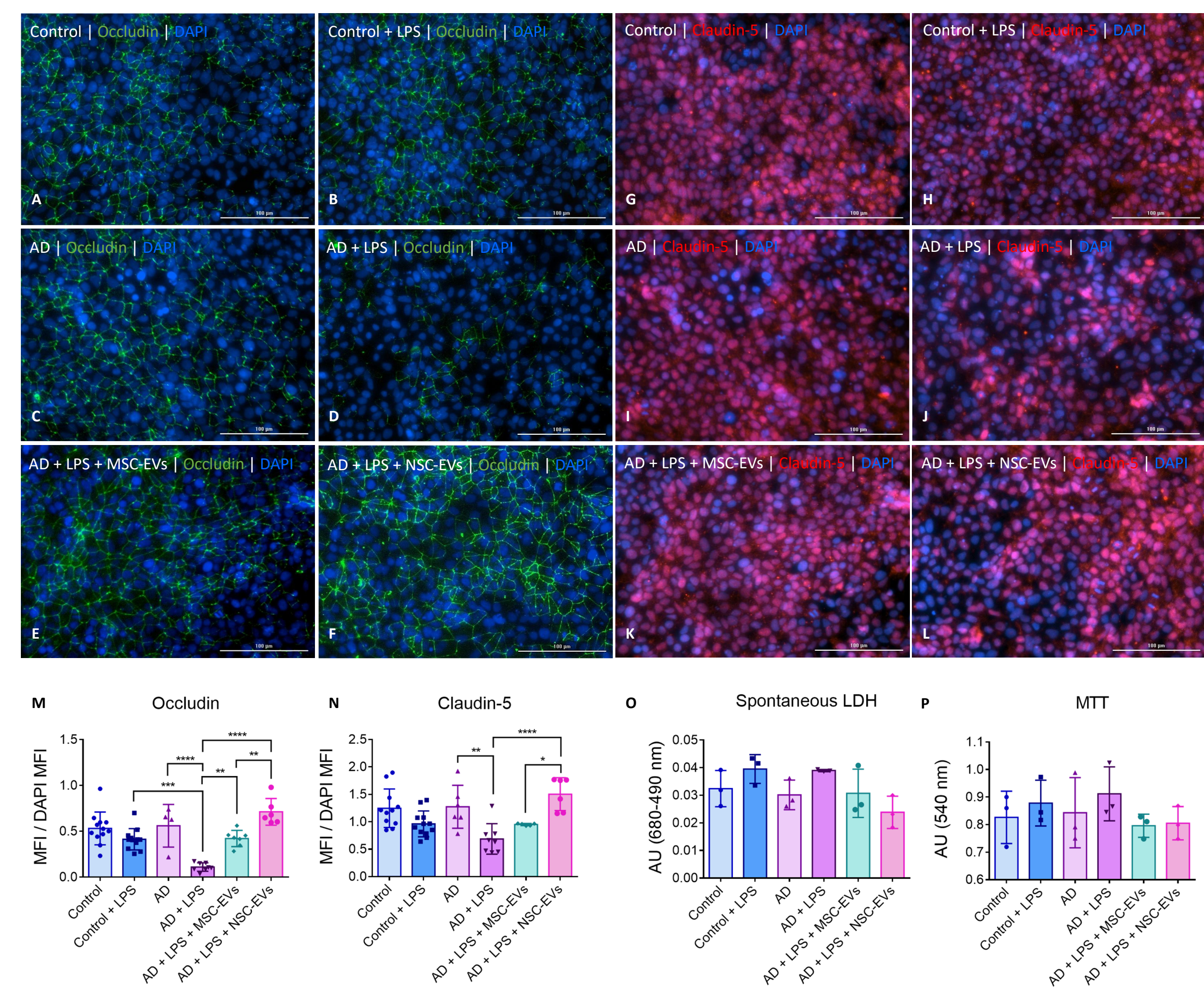


Figure 4. ICC images of control and AD BECs with or without LPS and MSC- and NSC-EVs stained for Occludin (A-F), and Claudin-5 (G-L). Mean fluorescence intensity (MFI) normalized to DAPI (M, N) was lower for AD + LPS and restored by MSC-EVs and NSC-EVs. Spontaneous LDH (O) and MTT (P) assays showed no differences in cell viability.

Conclusions

- MSCs produce a higher yield of EVs with higher concentrations of neurotrophic, survival, and immunomodulatory proteins
- AD (PSEN2) BECs had lower resilience to LPS compared to controls, showing reduced expression of tight junction proteins Occludin and Claudin-5 after 24 h of exposure
- Stem cell-derived EVs could restore tight junction protein expression with LPS exposure, with NSC-EVs having a greater effect
- LPS induced an inflammatory-like response in BECs, partially restored by MSC-EVs