# MODELING NEUROINFLAMMATORY DISEASES WITH IPSC-DERIVED MICROGLIA

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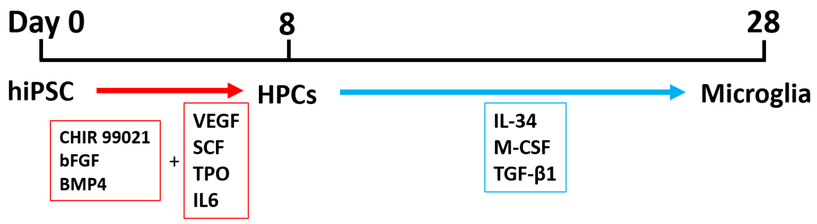
## Introduction

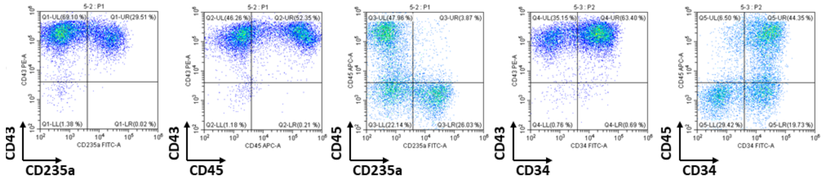
Microglia contribute to a range of neuroinflammatory and neurodegenerative disorders, but scientists have long suffered from a lack of quality *in vitro* models for these cell types. We have developed a robust and scalable method to produce highly pure, functionally validated, and ready-to-use microglia from human induced pluripotent stem cells (iPSC). These microglia show greater than 97% purity, as measured by immunostaining for IBA1 and TMEM119. These cells can be cryopreserved, thawed, and cultured in defined maintenance media with or without astrocytes or neurons for prolonged culture.

We then performed high-content imaging and an algorithm developed by PhenoVista Biosciences for quantification and phenotypic characterization of this neuroinflammation model. By treating the microglia with stimulants nigericin (Ngc) or ATP, we induced inflammasome formation. More specifically, we observed a re-distribution of inflammasome proteins (NLRP3 and ASC) from being diffuse and cytoplasmic to organized, punctate structures. This inflammasome formation could be blocked by the inhibitor MCC950, as indicated by decreased detection of ASC and NLRP3 puncta. Caspase-1 inhibition by treating the microglia with VX-765 also reduced the number of ASC puncta, though NLRP3 levels not decreased. These data show functionally relevant and measurable phenotypes in these human iPSC-derived microglia may be suitable for modeling neuroinflammatory diseases and for use in drug-screening assays.

## Differentiation of Human iPSCs to Microglia

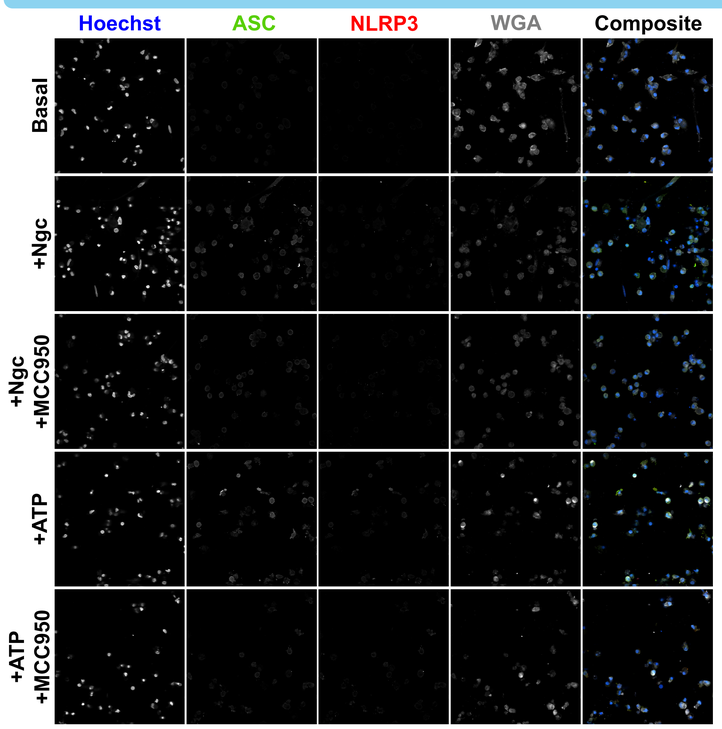


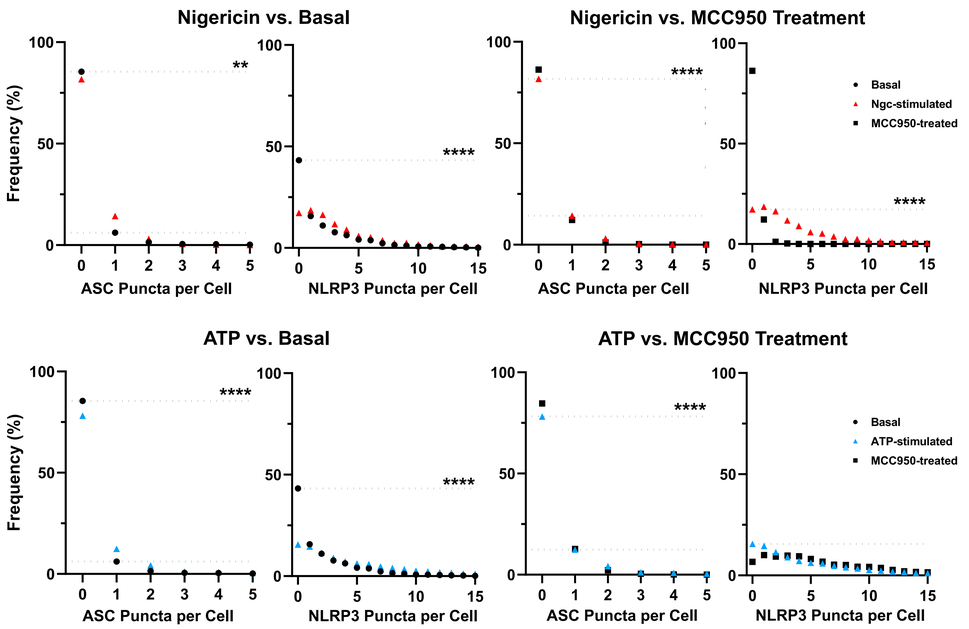

Hematopoietic progenitors were plated on Matrigel-coated flasks with IL-34, M-CSF, and TGF-β1 in serum-free conditions for 3 weeks. During differentiation of human IPSC to microglia, culture media was changed every 3-4 days. To induce mesoderm, cells were treated with CHIR99021, bFGF, and BMP4 for 2-3 days. To induce hematopoietic progenitors, cells were treated with VEGF, SCF, TPO, and IL-6 for an additional 5-6 days.

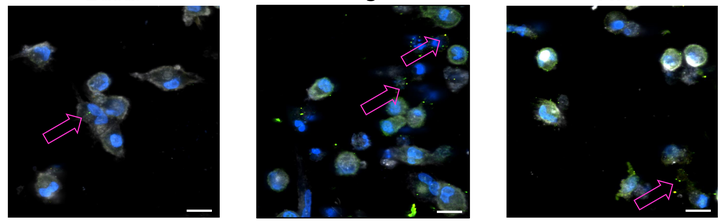



On day 8, HPCs were harvested, and their purity was confirmed by staining for CD34, CD43, and CD45. After 3 weeks of culture, the presence of microglia-specific markers was confirmed, including CD45, CD11b, CD33, CD115, CX3CR1, IBA-1, P2RY12, TMEM119, and TREM2.

## Stimulation upregulates ASC and NLRP3 in microglia, and inflammasome formation is blocked by MCC950 treatment.



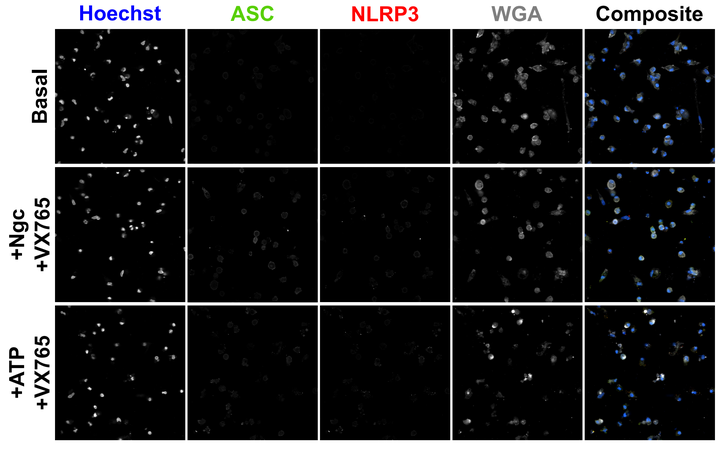



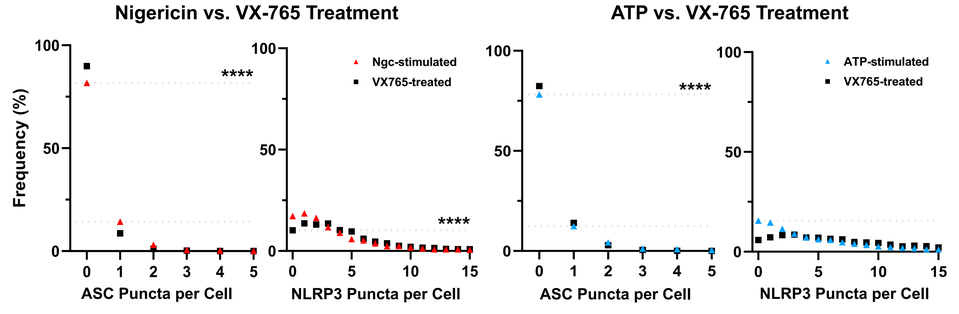

Left/top images: Treatment of microglia with stimulants nigericin or ATP induces inflammasome formation, as indicated by increased levels of punctate staining of inflammasome markers ASC and NLRP3. Staining for markers is reduced with treatment of cells with MCC950.
Scale bars indicate 25µm.
Left/bottom images: Composite images of stained microglia that were unstimulated (left), nigericin-treated (left) and ATP-treated (right). Scale bare indicates 25 µm.
Stains: Hoechst for nuclei; ASC and NLRP3 for inflammasome markers; WGA for whole-cell morphologies.
⇨ Arrows point to characteristic puncta that were identified in a PhenoVista algorithm for quantitative analysis.
Above graphs: Quantification of number of puncta per cell shows increased levels with nigericin stimulation. More moderate effects are seen with ATP stimulation. Treatment of microglia with MCC950 blocks inflammasome formation, as shown by decreased numbers of puncta per cell. Mann-Whitney test was performed on data sets that passed QC ($p<0.01$ **, $p<0.0001$ ****).

**Compared to unstimulated, control cells, brighter and higher levels of punctate staining are observed in stimulated cells, indicating induction of inflammasome formation, which can be blocked with MCC950 treatment.**

## Caspase inhibition by VX-765 blocks inflammasome formation in microglia.

**Inhibition of caspase-1 by treatment with VX-765 reduced inflammasome formation in microglia treated with nigericin or ATP, though not as effectively as treatment with MCC950. VX-765 reduced levels of ASC staining but did not affect NLRP3 levels. Mann-Whitney test was performed on data sets that passed QC ($p<0.0001$ ****).**




## Summary

- We produced highly pure iPSC-derived human microglia in a serum-free, feeding-free, and sorting-free system.
- Inflammasome formation was induced by treating microglia with nigericin or ATP, as indicated by staining for ASC and NLRP3 inflammasome markers.
- MCC950 blocks inflammasome formation induced by nigericin or ATP.
- VX-765 treatment of microglia reduced levels of ASC but did not affect NLRP3 levels.
- Our protocol and imaging assay may be a useful model of neuroinflammatory diseases for future work.