

Microglia Expressing the Alzheimer’s Disease Protective Variant *PLCγ2*-P522R Suppress the NLRP3 Inflammasome and Mitochondrial Stress

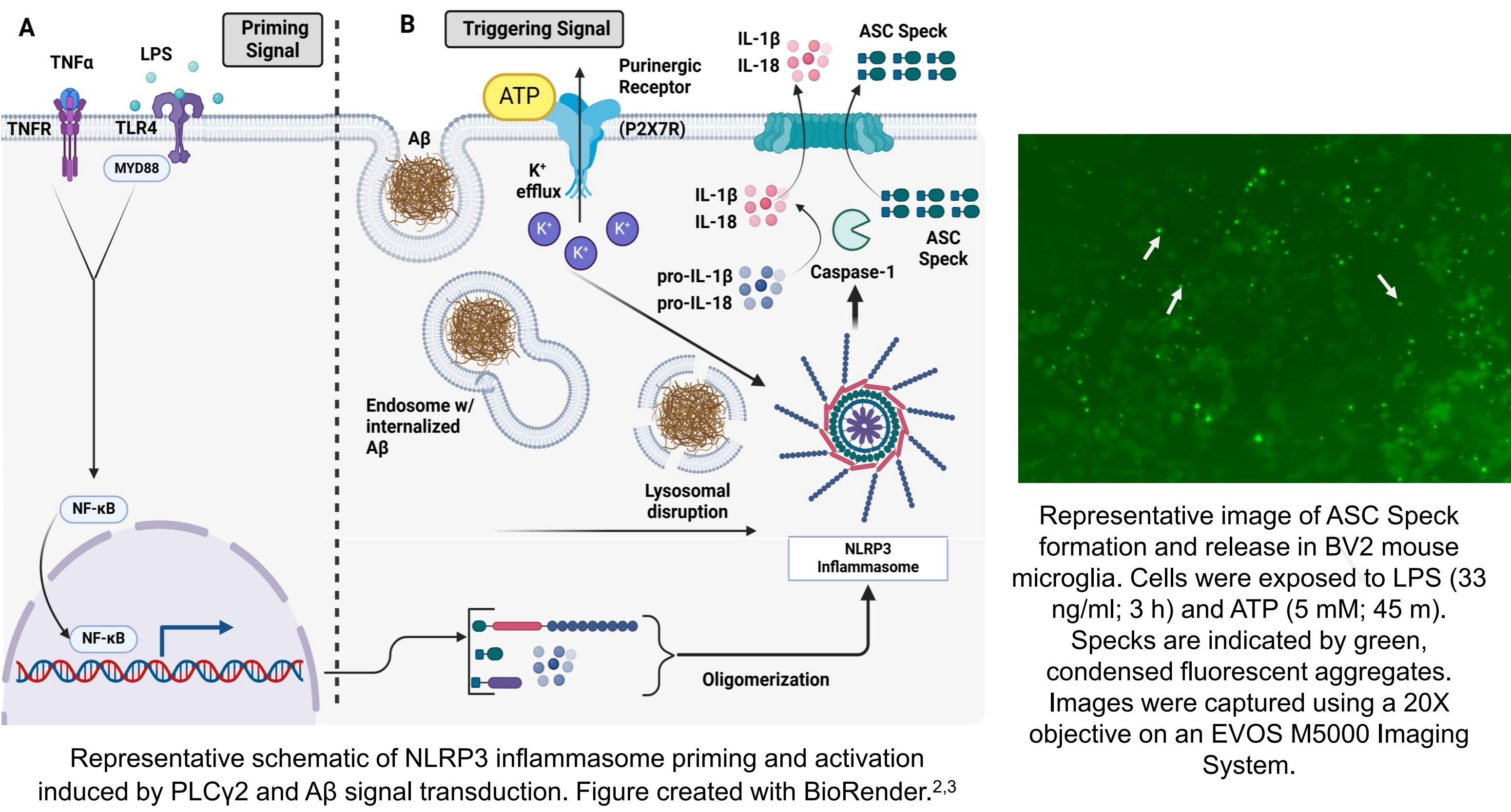
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Background

- Microglia, the brain resident immune cell, serve critical roles in clearing aberrant proteins and in orchestrating the inflammatory response to disease or brain injury.
- Pathological hallmarks of Alzheimer’s disease (AD) [amyloid-beta (Aβ) plaques and widespread neuroinflammation] are associated with microglial dysregulation^{1,4,5}.
- GWAS studies have identified multiple AD risk and protective variants located in genes expressed by microglia.
- The P522R variant of the Phospholipase C gamma 2 (*PLCγ2*) gene is uniquely expressed by microglia in the CNS and is protective against AD⁴.
- PLCγ2* cleaves the phospholipid PIP₂ into IP₃ and DAG, which increase cytosolic Ca²⁺ levels and activate inflammatory pathways.
- PLCγ2*-P522R mice have a reduced plaque burden and differential transcription profile of genes associated with inflammation and the endo-lysosomal pathway⁴.

The NLRP3 Inflammasome

- The NOD-like Receptor (NLR) inflammatory signaling pathway leads to the generation of NLRP3 inflammasome complexes.
- The *PLCγ2* pathway interacts with NLR signaling through the ability of NLRP3 to sense intracellular stress and danger-associated molecular pattern (DAMP) receptor signals^{3,4}.
- The NLRP3 inflammasome requires a two-part activation: NF-κB transcriptional priming followed by oligomerization of NLRP3, caspase-1, and ASC into the NLRP3 inflammasome complex.
- Activated NLRP3 inflammasomes induce the release of pro-inflammatory cytokines (IL-1β and IL-18) and the ASC speck.
- Microglia derived from AD patients display enhanced transcriptional activation of the NLR pathway and mediators of the NLRP3 inflammasome relative to control patients⁵.
- ASC speck-Aβ oligomer complexes are seen in post-mortem brain tissue from AD patients¹.

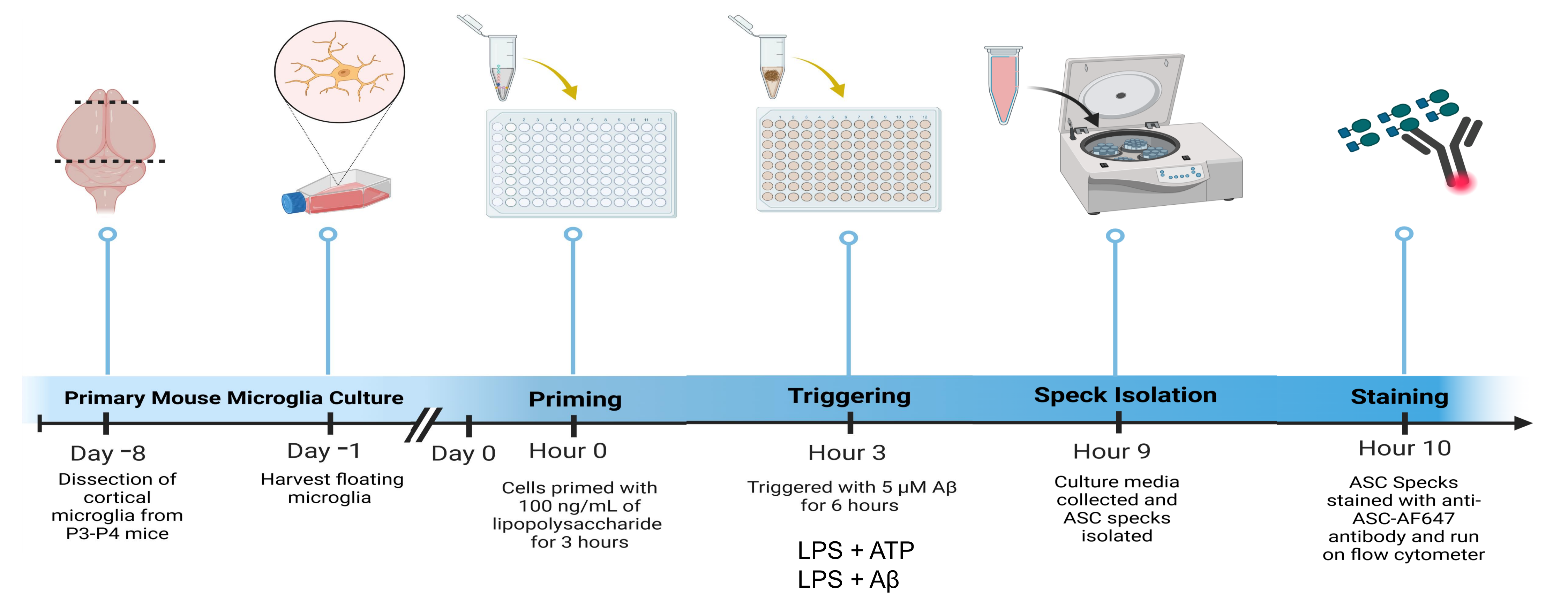


Objectives

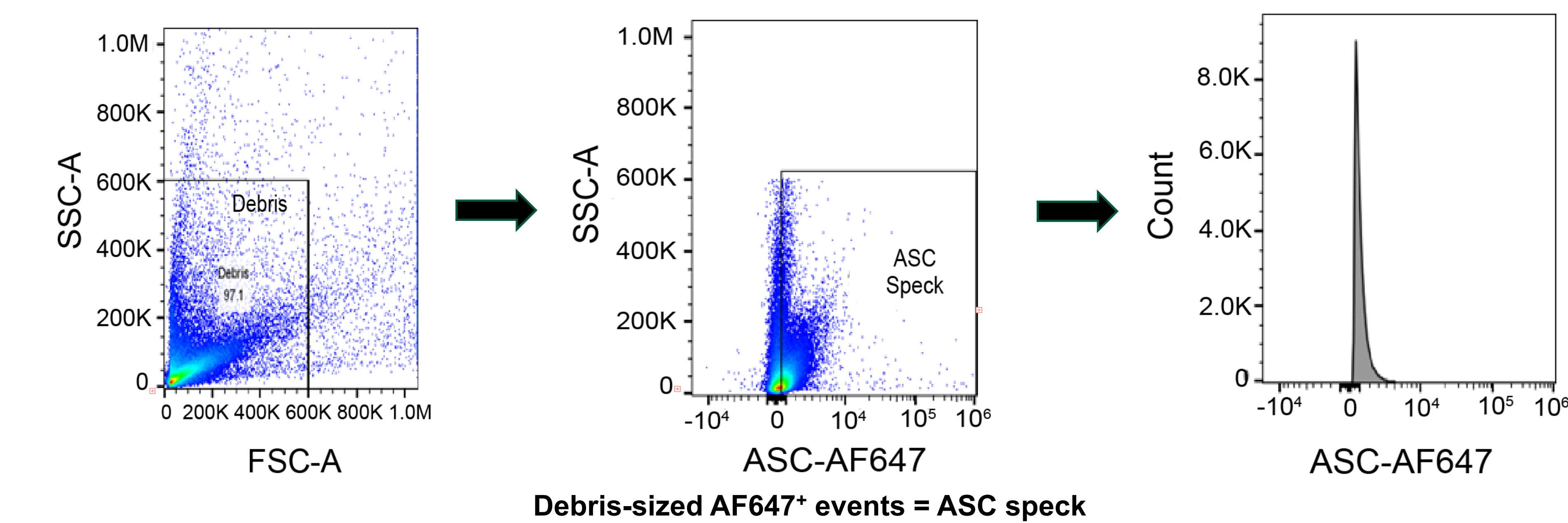
P522R has been shown to enhance the phagocytic capacity of microglia to uptake Aβ⁴. However, *PLCγ2* and its synergism with the NLRP3 inflammasome can also drive inflammatory dysregulation and potentially Aβ aggregation. Taken together, my hypothesis is that microglia with the P522R variant exert their protective effects through reduced NLRP3 inflammasome activation. This reduction in activation could result from a greater stabilization of mitochondrial bioenergetics in response to Aβ. Using flow cytometry and immunofluorescence for the ASC Speck, in addition to a flow cytometry-based assay for mitochondrial membrane potential, this hypothesis will be tested via the following aims:

- Aim 1:** Compare the activation of the NLRP3 inflammasome in WT and P522R^{+/+} primary mouse microglia using a canonical inflammasome activation paradigm.
- Aim 2:** Assess whether the P522R variant influences NLRP3 inflammasome activation in response to Aβ.
- Aim 3:** Evaluate whether modulation of mitochondrial oxidative phosphorylation could serve as an upstream mediator for NLRP3 induction.

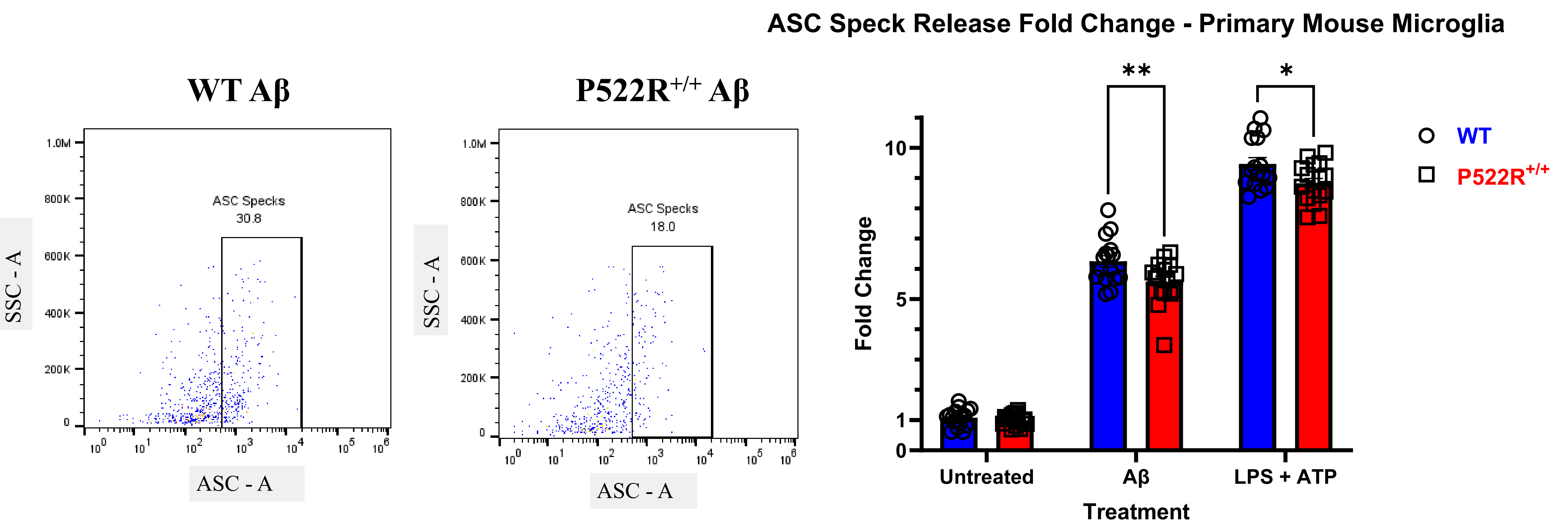
Methods and Experimental Timeline



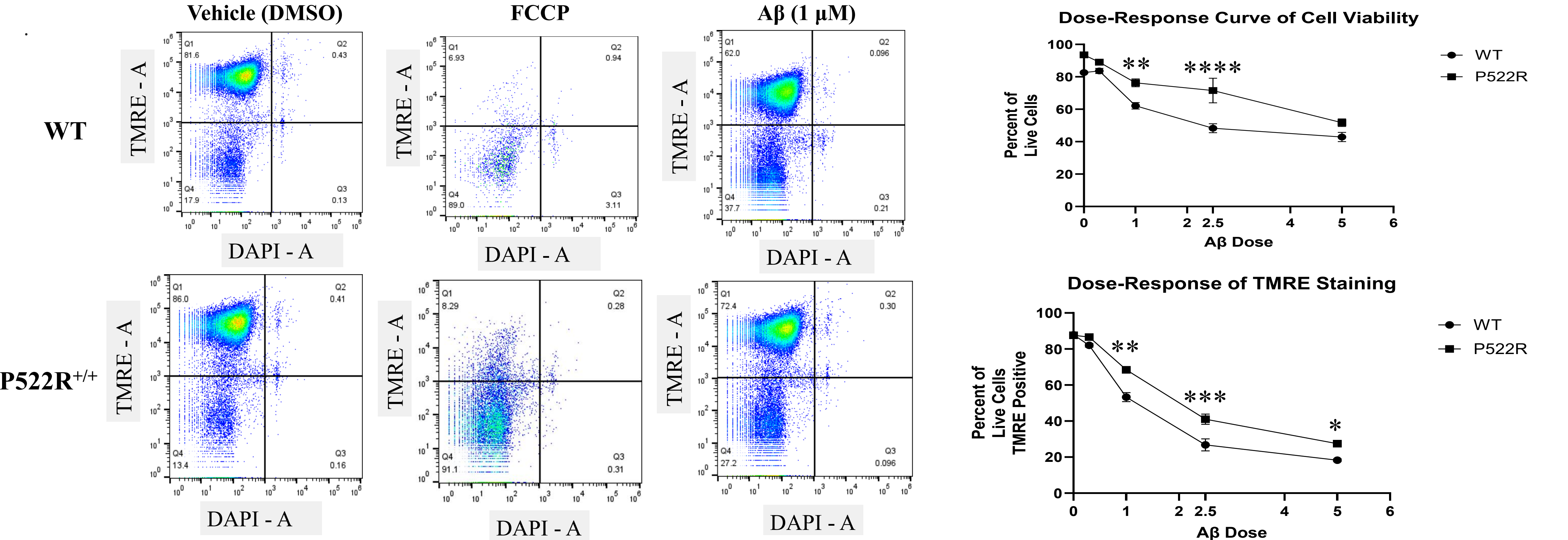
Flow Cytometry Analysis of Secreted ASC Specks



FlowJo pipeline for Secreted ASC Speck Quantification. Primary mouse microglia were stimulated with LPS + ATP. Collected ASC Specks were run through an Attune NxT flow cytometer. Debris-sized events that were positive for AF647 were counted as an ASC Speck. Area under the histogram was performed to calculate the total number of released specks. Figures were created in FlowJo.

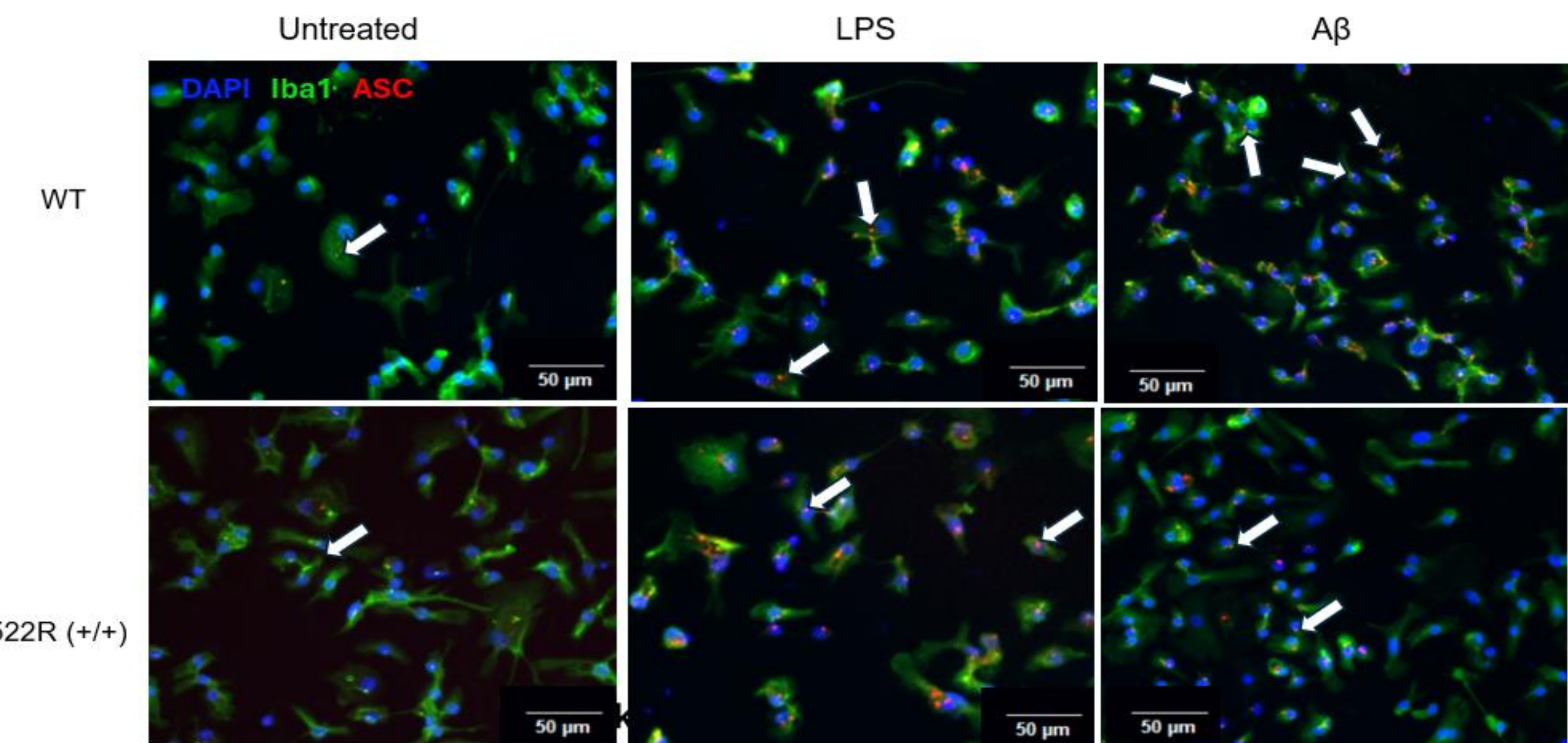


Microglial Cell Viability and Mitochondrial Membrane Potential Analysis



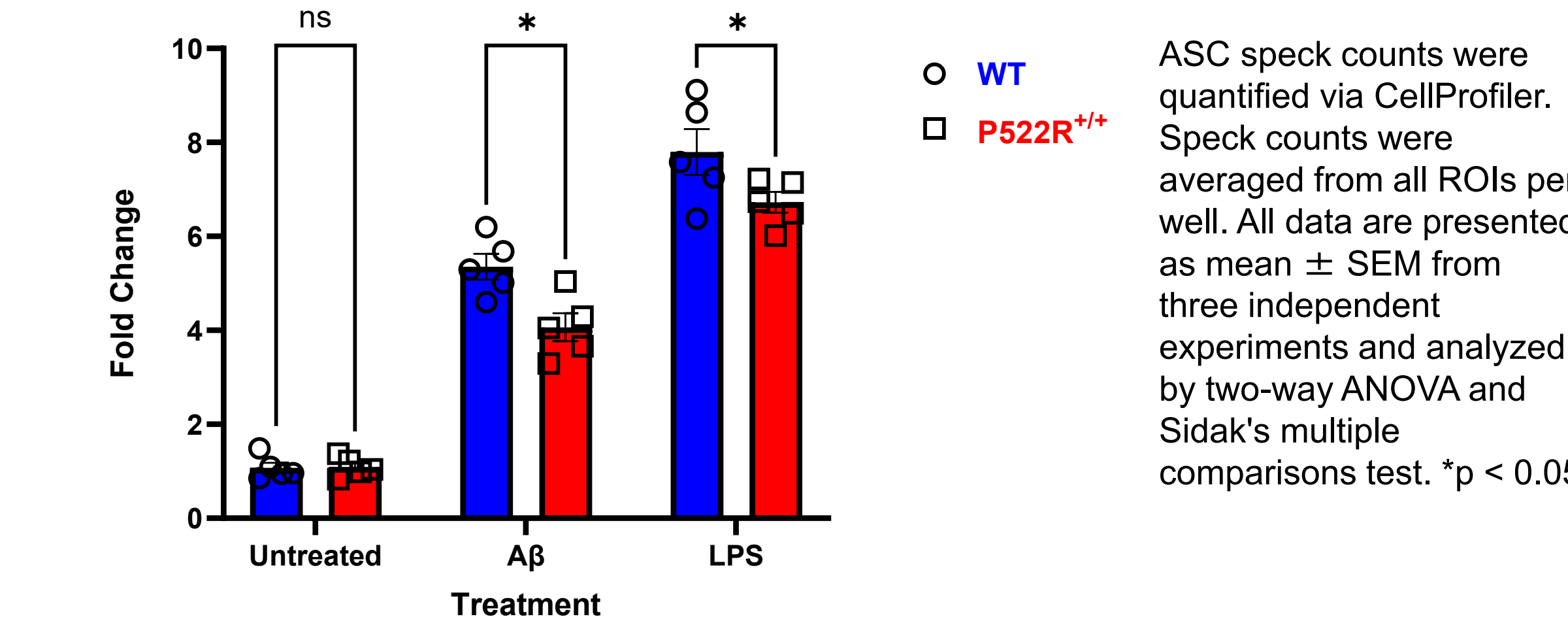
ASC Speck Immunofluorescence Paradigm

- The lowered release of ASC speck particles could be attributed to a reduced production of endogenous ASC or to an inhibition of ASC speck release once fully oligomerized.
- We designed an immunofluorescence assay that enables us to analyze the intracellular formation of ASC specks.
- We wanted to assess whether ASC speck production significantly differed between WT and P522R^{+/+} primary mouse microglia.
- Cells were fixed 3 hours after being exposed to Aβ and stained for ASC (red), Iba1 (microglia marker; green), and DAPI (nuclei marker; blue).



Representative images of ASC specks (red) residing in the perinuclear space of microglia (Iba1; green). DAPI (blue) represents nuclei. Images were captured from 7 ROIs per well (n=3) at 20X on an IN Cell Analyzer 2200. Scale bars represent 50 μm.

ASC Speck Formation Fold Change - Primary Mouse Microglia



Summary and Future Directions

- The P522R variant of *PLCγ2* reduces pro-inflammatory signaling secondary to Aβ exposure in mouse microglia.
- We developed two novel methods for assaying NLRP3 inflammasome activity in primary mouse microglia.
- Using these assays, we observed that P522R^{+/+} microglia suppressed NLRP3 inflammasome activation in response to Aβ and ATP.
- P522R^{+/+} microglia demonstrate reduced ASC speck formation and release.
- Relative to WT microglia, P522R^{+/+} microglia exhibited a dose-dependent increase in mitochondrial stability and cell viability when exposed to Aβ.

- We plan to assess whether small-molecule activators of *PLCγ2* recapitulate the impact of the P522R variant on NLRP3 inflammasome activation and mitochondrial bioenergetics.

References

