Title: Potential Conversion of Soluble Amyloid-Beta into Insoluble Forms in Microglial Lysosomes

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Abstract: The propagation of amyloid beta plaques is central to Alzheimer's disease (AD) progression. Many AD risk factors correlate with an increase in soluble Aβ species, but how these are converted into plaques is unknown. Genetic risk factors implicate microglia, the brain's resident immune cells, as a critical cell type in AD. In late stages of Aβ progression, microglia work to halt the toxic effects of plaques, but during initial deposition, their ability to take up soluble Aβ is necessary for plaque formation. Because of the potentially aggregation-hospitable environment of lysosomes, **I hypothesize that microglial lysosomes convert soluble Aβ into insoluble aggregates.** While cellular and *in vivo* studies have struggled to pinpoint this aggregation, I am testing the effects of modulating lysosomal conditions and quantifying changes on a dual cellular-structural biology level. This unique approach uses both classical means of modulating lysosomal function and quantifying dense aggregate formation via advanced techniques of cellular cryo-electron tomography (cryo-ET) to ground findings in structural certainty. I test whether lysosomal acidity and proteolytic function in lysosomes mediate dense aggregate seeding within microglia by selectively exposing microglia to specific Aβ plaque species (monomers, oligomers, nanofibrils, and aggregates) and using lysosomal inhibitors to enhance or discourage aggregation within lysosomes. Aβ aggregation and accumulation is analyzed by confocal microscopy and ELISA, as well as high-resolution cellular fast ion beam (FIB)-milling cryo-ET.

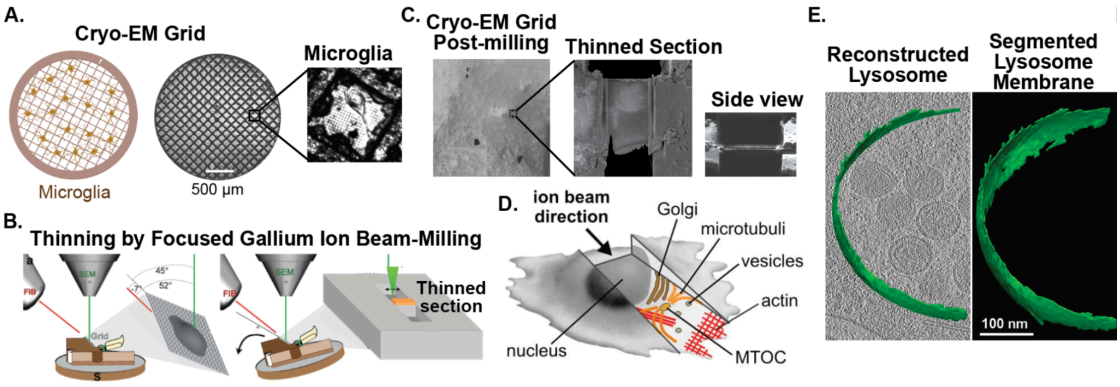

Figure. Focused ion beam transmission electron microscopy reveals subcellular structural details. A. schematic of microglia on a cryo-EM grid. Middle: transmission image of a frozen cryo-EM grid of BV2 cells, black square highlighting single microglia. **B.** Schematic explaining focused ion beam (FIB) milling from Singh & Villa, 2024. **C.** Left: scanning electron microscope image of a FIB-milled grid, with a black square highlighting a single thinned section. **D.** Schematic of a thinned cell from Rigort et al. 2012. **E.** 2D slice of reconstructed tomogram volume of a microglial lysosome, with voxel-segmented membrane.