



Harnessing Human iPSC-Microglia to target Amyloid- β and Tau Pathology *in vivo*



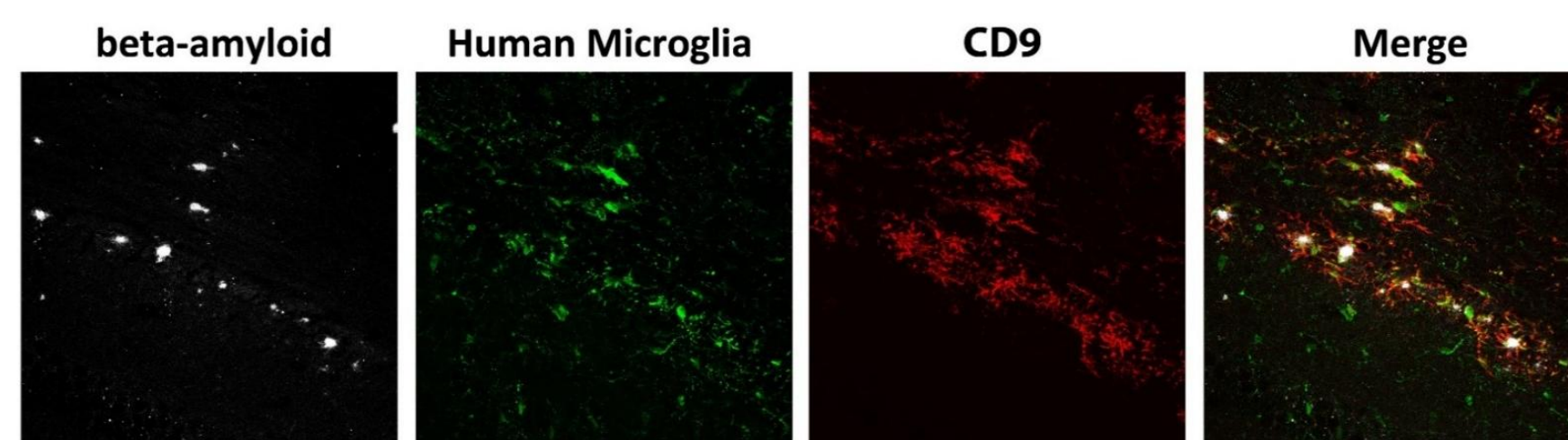
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Introduction

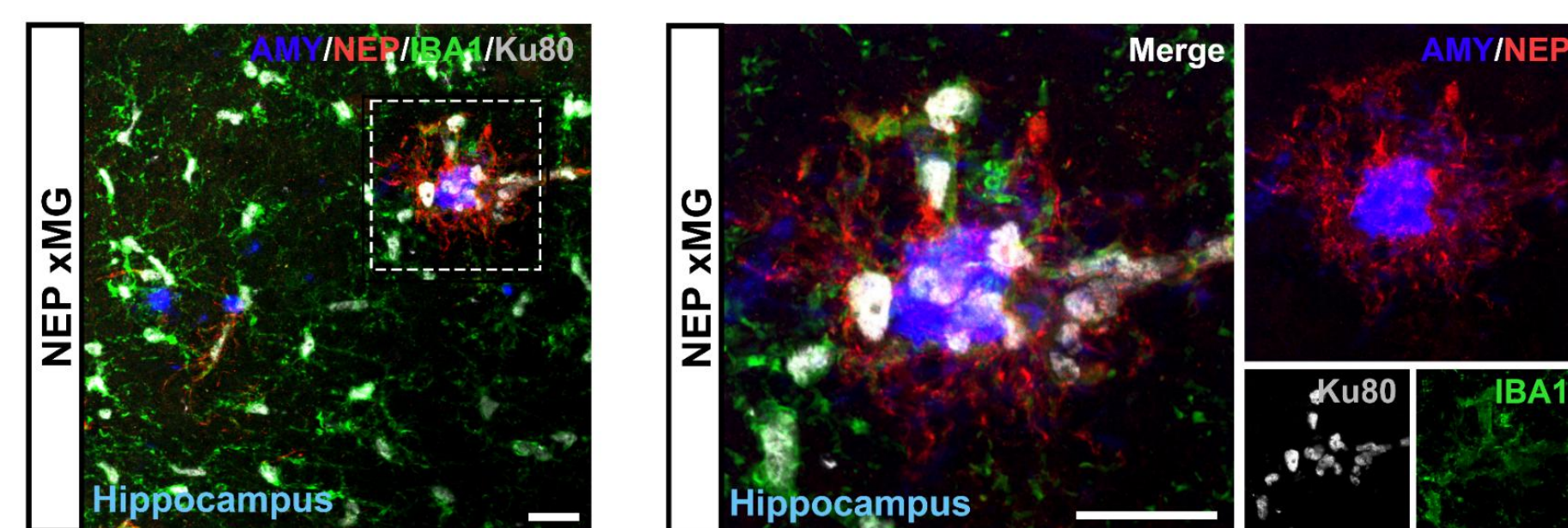
Xenotolerant mouse models of amyloid and tau pathology are increasingly being used to provide important insight into the genetic risk factors that underlie AD. More recently, these models are being employed to examine the impact of genetically-modified human microglia on disease pathology. To examine the potential utility of microglia cell therapies we examined two complimentary approaches. One method utilizes iPSC-microglia that were modified to express the amyloid-degrading protease neprilysin under control of a plaque-responsive promoter, providing targeted delivery of a therapeutic protein. A second method utilizes a constitutive microglial promoter to drive expression of an anti-amyloid chimeric antigen receptor (CAR). The impact of these approaches on AD pathology was then examined using histological, biochemical, and transcriptomic approaches. Thus far our studies demonstrate that iPSC-microglia can provide a safe and effective approach to achieve widespread delivery of therapeutic proteins to the brain, while avoiding potentially detrimental effects of peripheral myeloid cell engraftment. Microglial mediated delivery of beta-amyloid targeted proteins leads to significant reductions in amyloid plaques and downstream pathologies. Ongoing studies are now further examining the impact of these approaches on a combination of amyloid and tau pathologies.

Microglial CD9 highly upregulated at sites of Amyloid Pathology



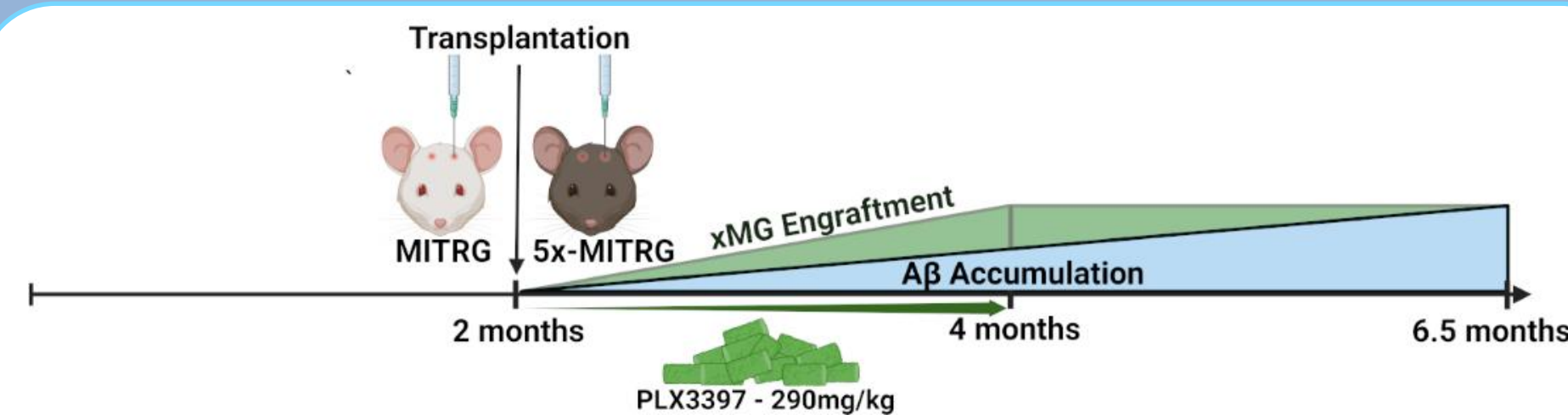
Single-cell sequencing of xenotransplanted microglia (xMGs) revealed altered population distributions and human disease associated microglial (DAM) responses to amyloid plaques (Hasselmann et. al., Neuron, 2019). Immunohistochemistry confirms the upregulation of CD9 in human xMGs at the protein level.

CD9 can drive delivery of the amyloid-degrading protease Neprilysin



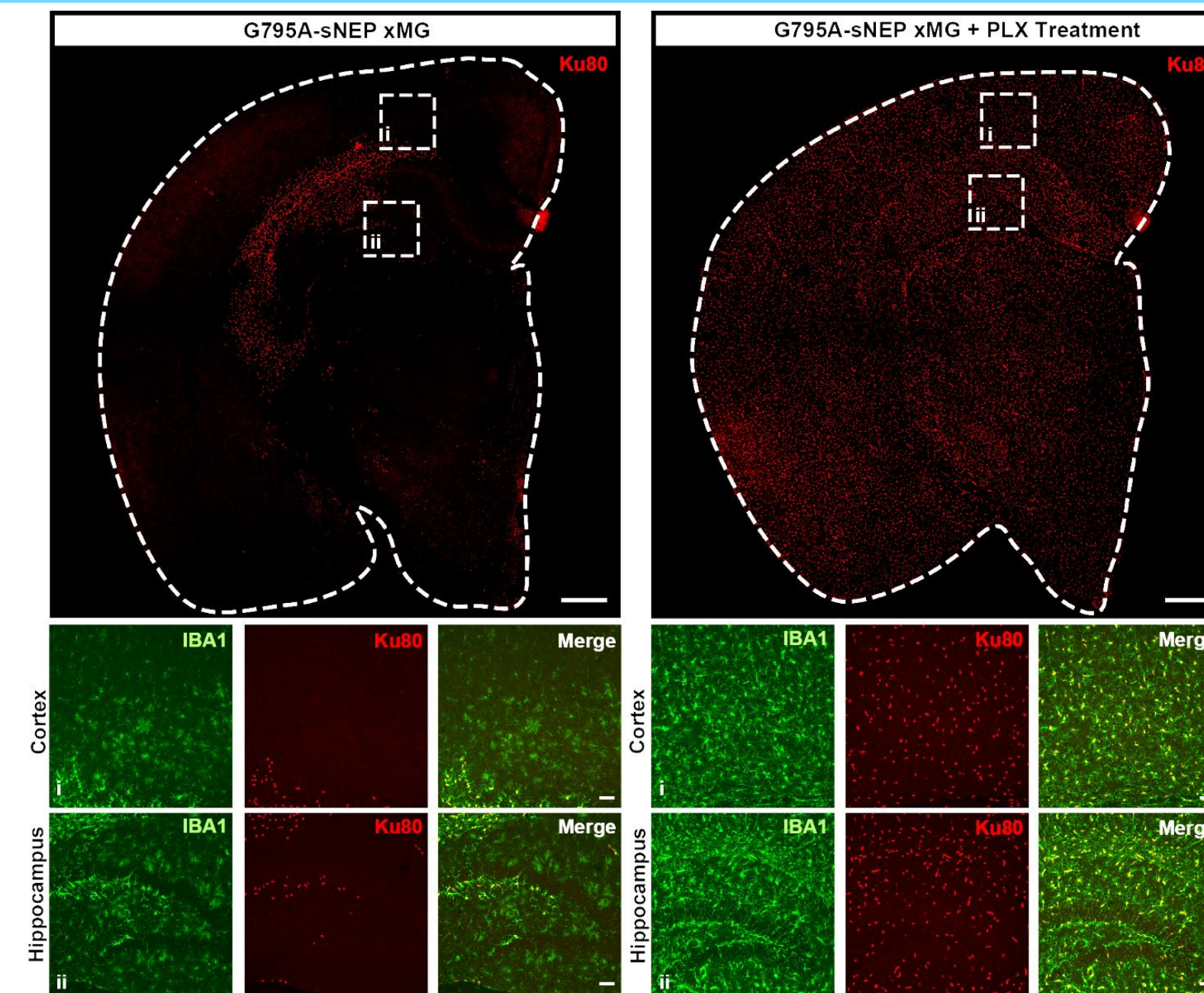
Genetic modification of the endogenous CD9 locus can facilitate delivery of neprilysin by xenografted iPSC-microglia (xMG) in response to amyloid depositions *in vivo*.

Transplantation Paradigm

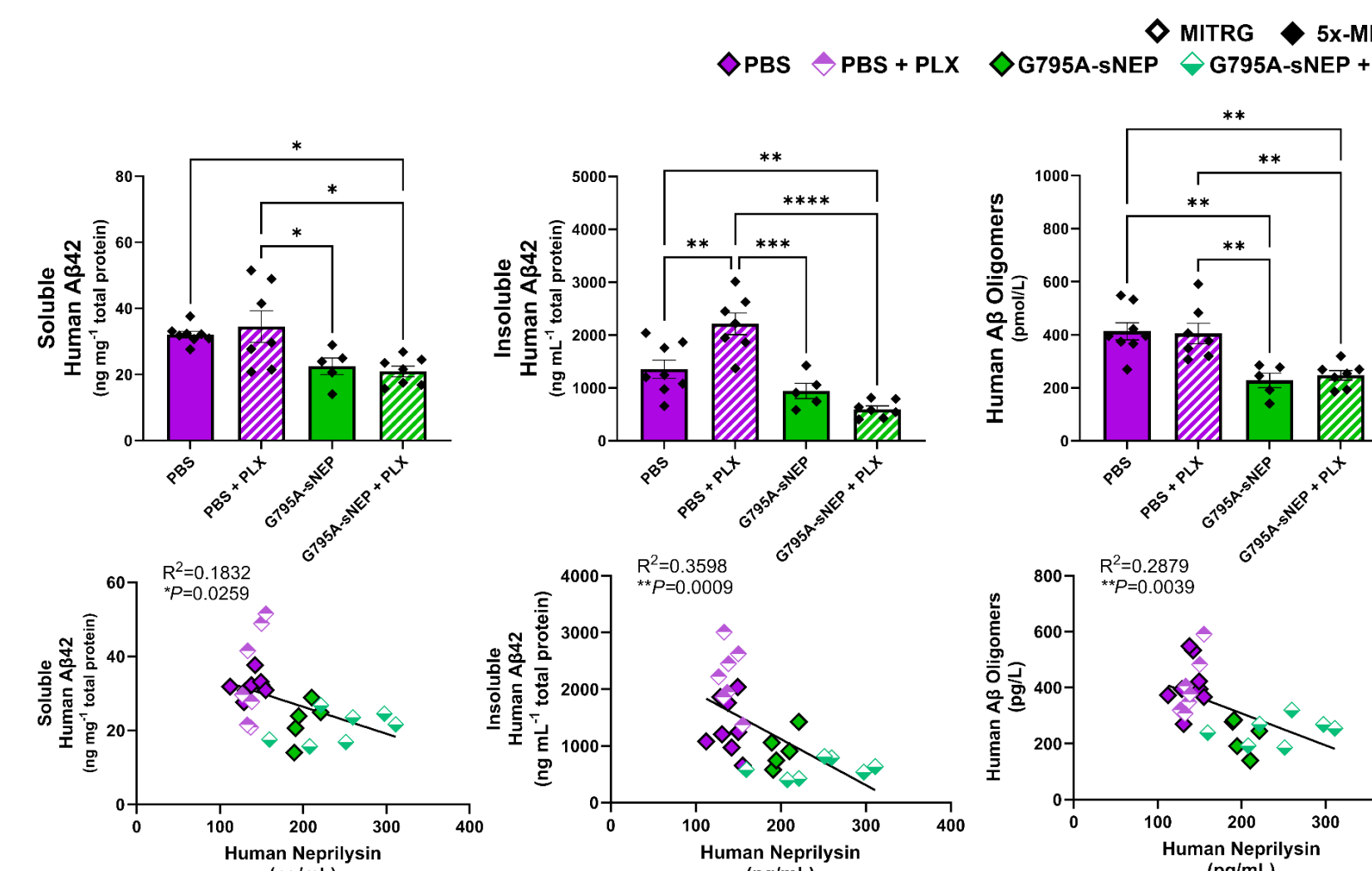


We previously identified a single amino-acid substitution in CSF1R (CSF1R-G795A) confers human microglial resistance to potent inhibitors *in vivo* allowing for the CNS-wide replacement of endogenous murine microglia. Therefore, patient-derived, human induced pluripotent stem cells (iPSCs) CRISPR engineered to produce secreted neprilysin (sNEP) under the endogenous CD9 promoter were further edited to introduce CSF1R-G795A. sNEP-G795A iPSCs were then differentiated into microglial progenitors (HPCs) and transplanted into xenotolerant MITRG and 5x-FAD-MITRG (5x-MITRG) transgenic mice at 2 months of age. Mice were then treated with or without PLX3397, a potent CSF1R inhibitor, for 2 months then fed regular chow *ad libitum* until termination. At 6.5 months, mice were sacrificed, and brains were examined via immunohistochemical and biochemical approaches.

Introduction of a G795A mutation within CSF1R enables brain-wide engraftment of sNEP xMG

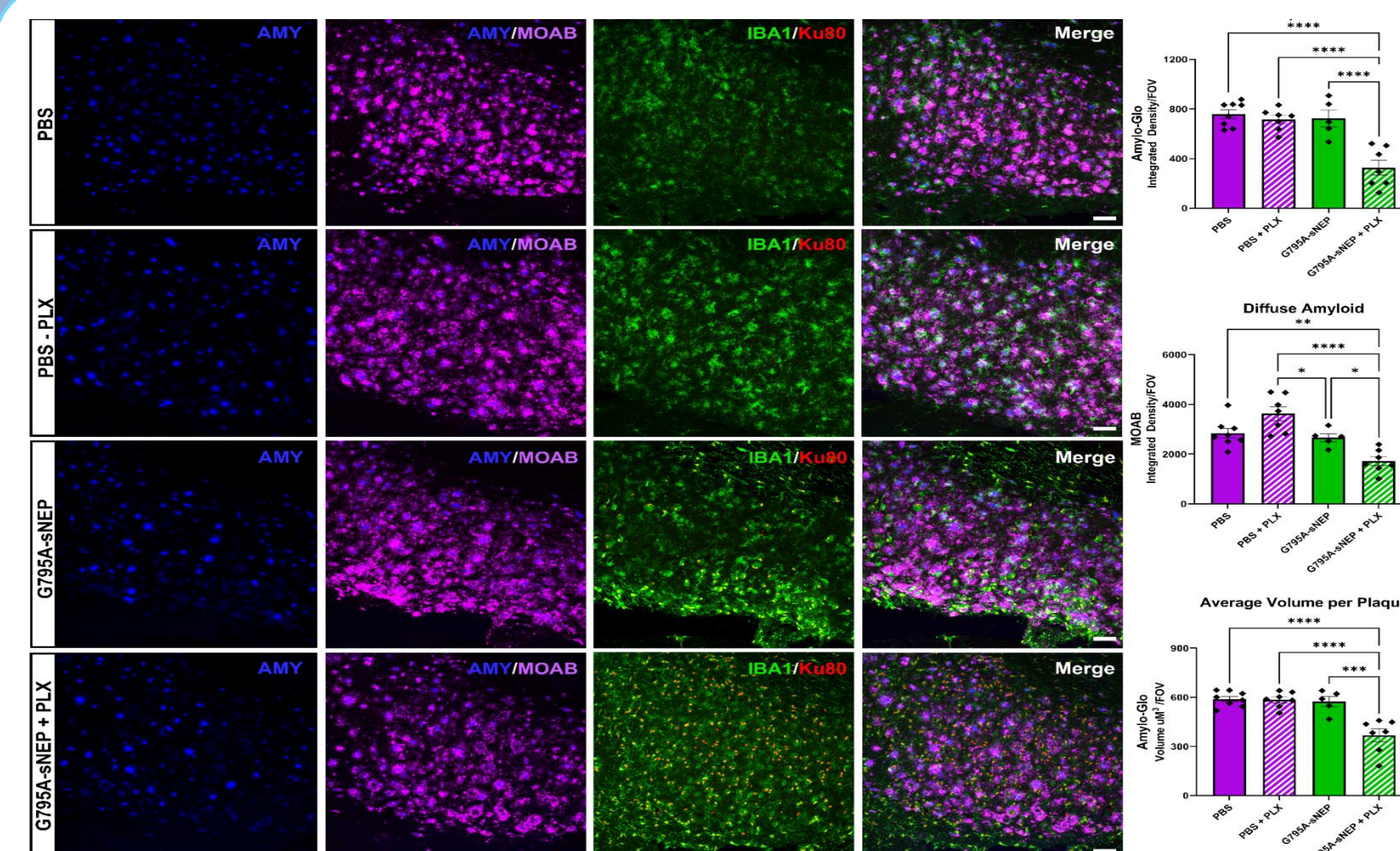


sNEP-microglia reduce soluble and insoluble levels of beta-amyloid



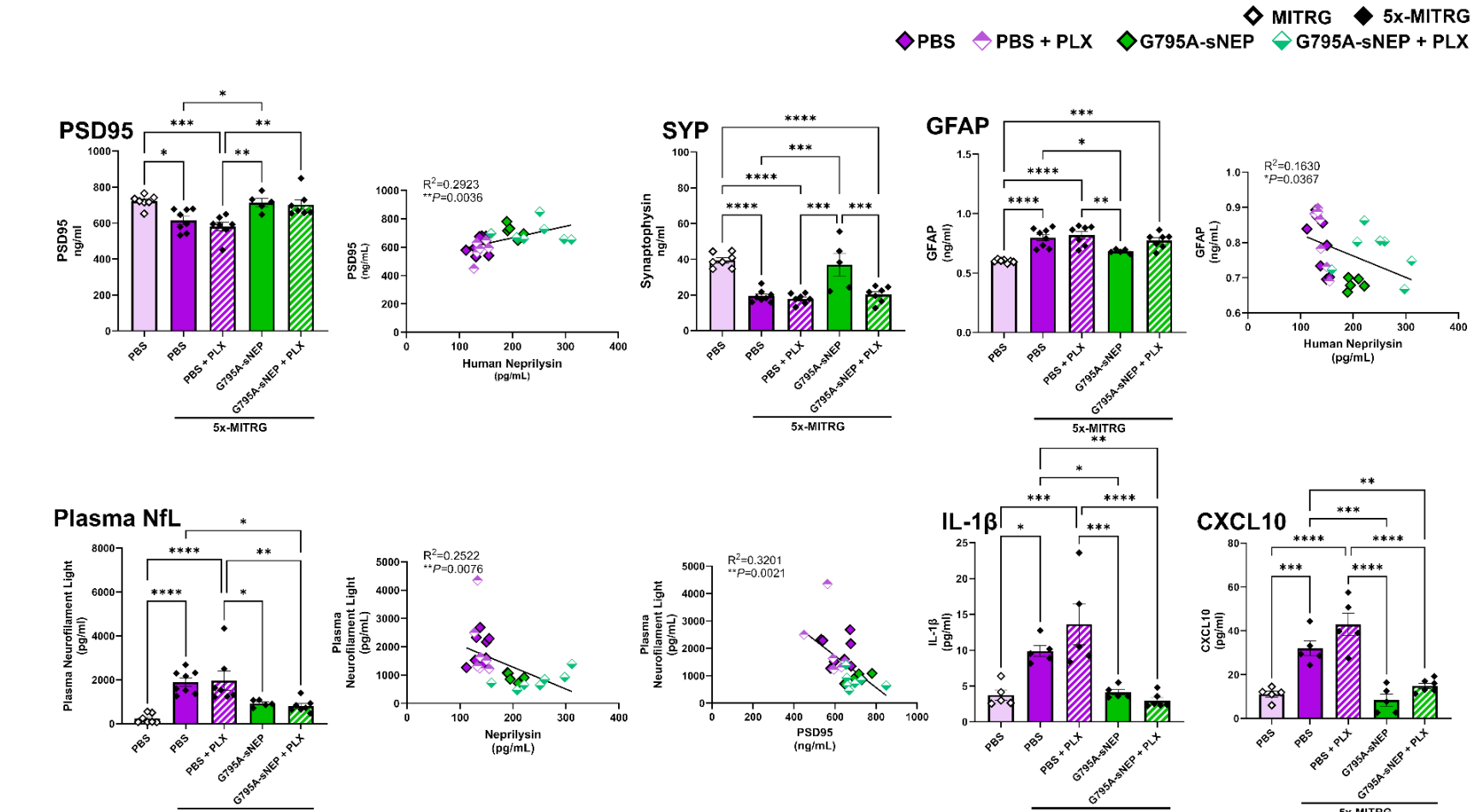
Biochemical analysis of soluble and insoluble samples reveals reduction in A β 42 and toxic A β oligomers correlate significantly with increased levels of microglial delivered sNEP in transplanted 5x-MITRG mice.

Pathology-responsive microglial delivery of neprilysin reduces amyloid burden within the plaque-dense subiculum



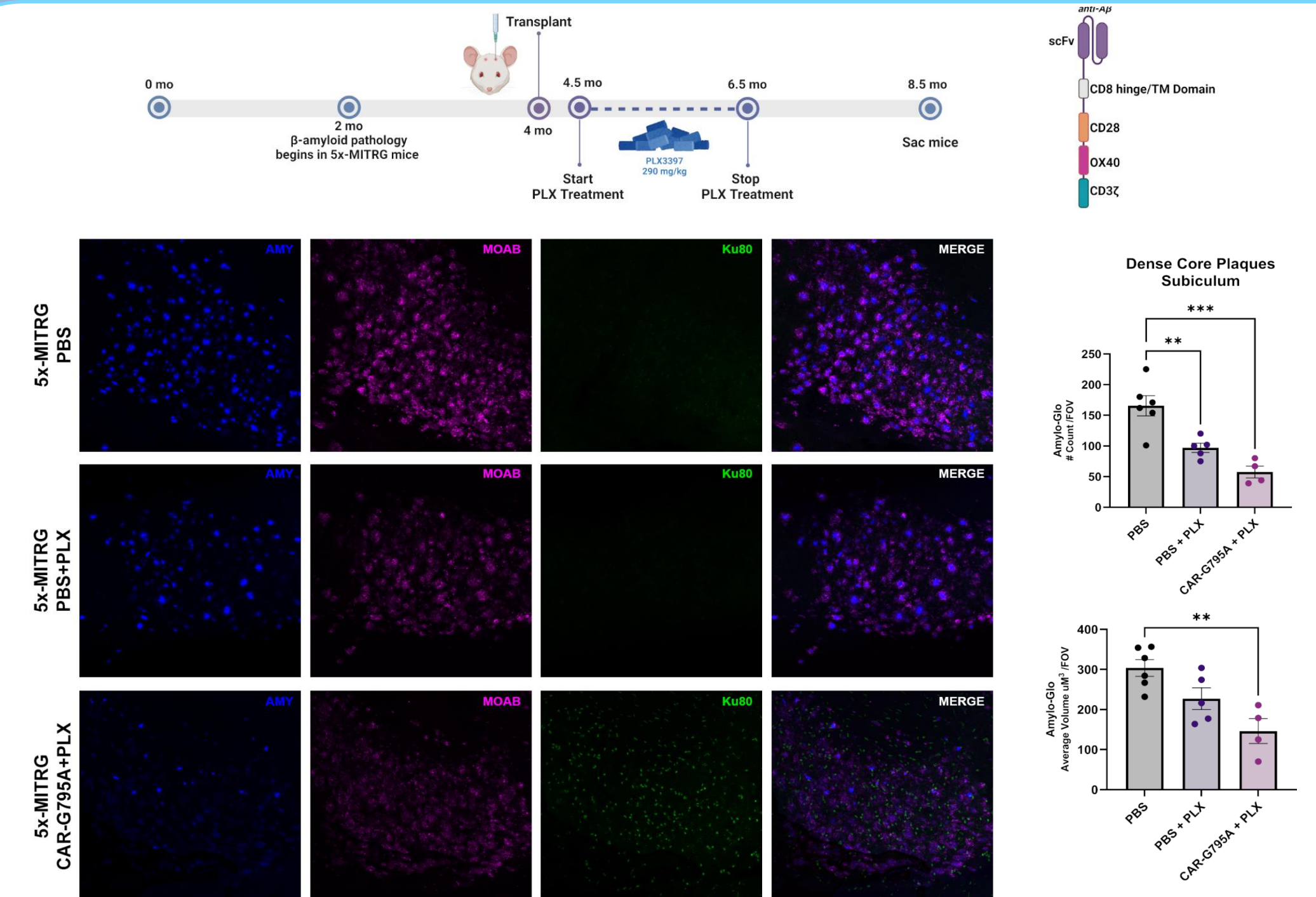
IHC analysis of compact (Amy, blue) and diffuse (MOAB, purple) amyloid within the subiculum of 5x-MITRG mice reveals significant reduction by G795A-sNEP xMG.

sNEP xMG reduce markers of neurodegeneration and inflammation



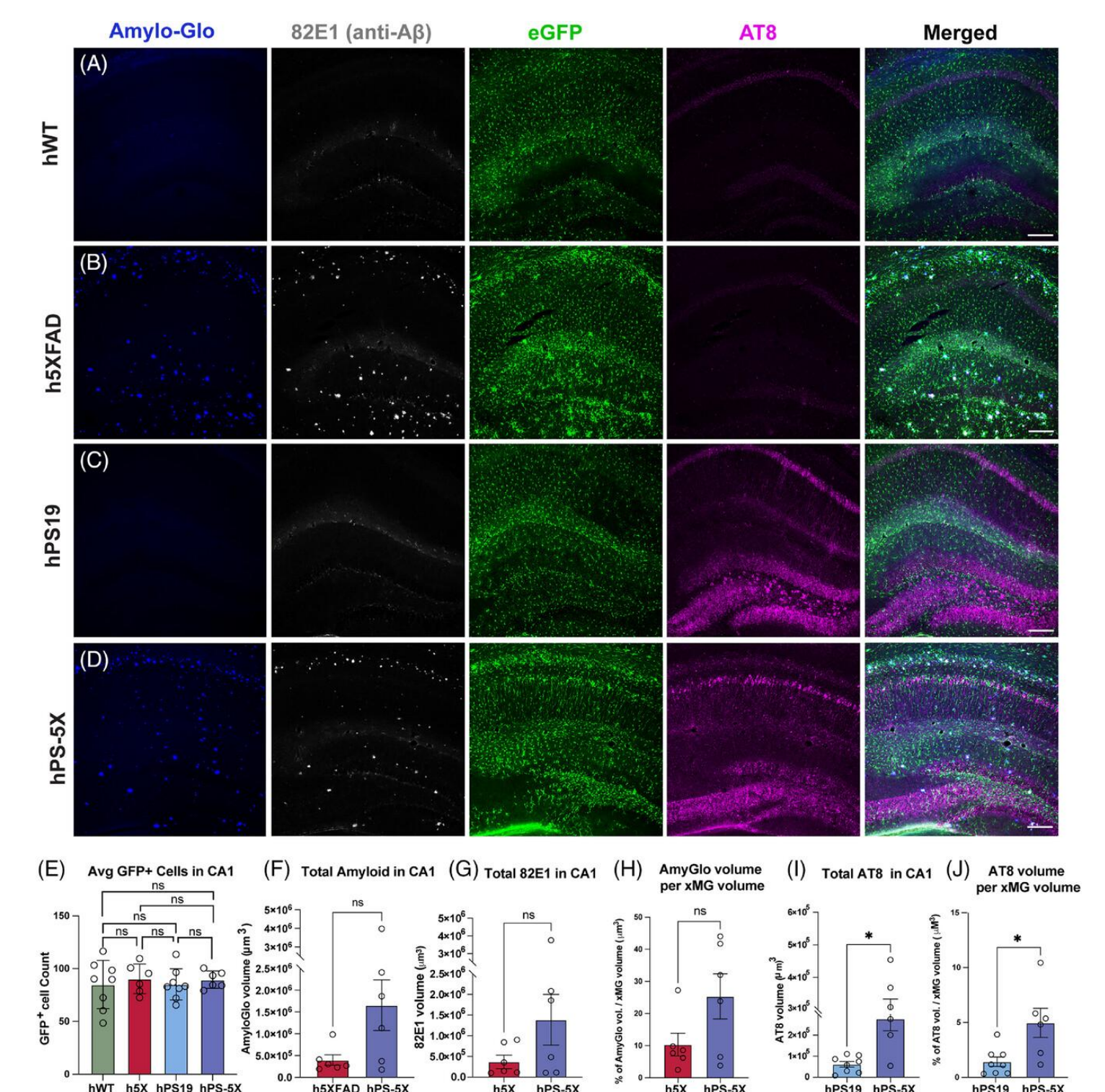
Microglial-mediated delivery of Neprilysin prevents synaptic degradation and reduces plasma NFL levels and markers for inflammation in transplanted 5x-MITRG mice.

Future Direction: Amyloid-targeting CAR iPSC-microglia



CSF1R-G795A expressing iPSCs we further modified via CRISPR gene editing to express an amyloid-targeting CAR construct under control of the endogenous IBA-1 (AIF1) promoter. Initial experiments suggest that brain wide delivery of CAR-xMGs reduce amyloid plaque load within the subiculum. Surprisingly, PLX3397 treatment alone between 4.5-6.5 months of age also partially reduced dense core plaque load.

We recently developed hPS-5X mice, a xenotolerant model of AD that develops both amyloid and tangle pathologies (Coburn, Eskandari-Sedighi, et. al., Alz & Dem, 2025). Similar to prior reports, we find that amyloid pathology accelerates the accumulation of hyperphosphorylated tau in xMG engrafted hPS-5X mice. Ongoing studies are now examining whether amyloid targeting CAR-microglia can reduce or delay the development of both of these hallmark AD pathologies.



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