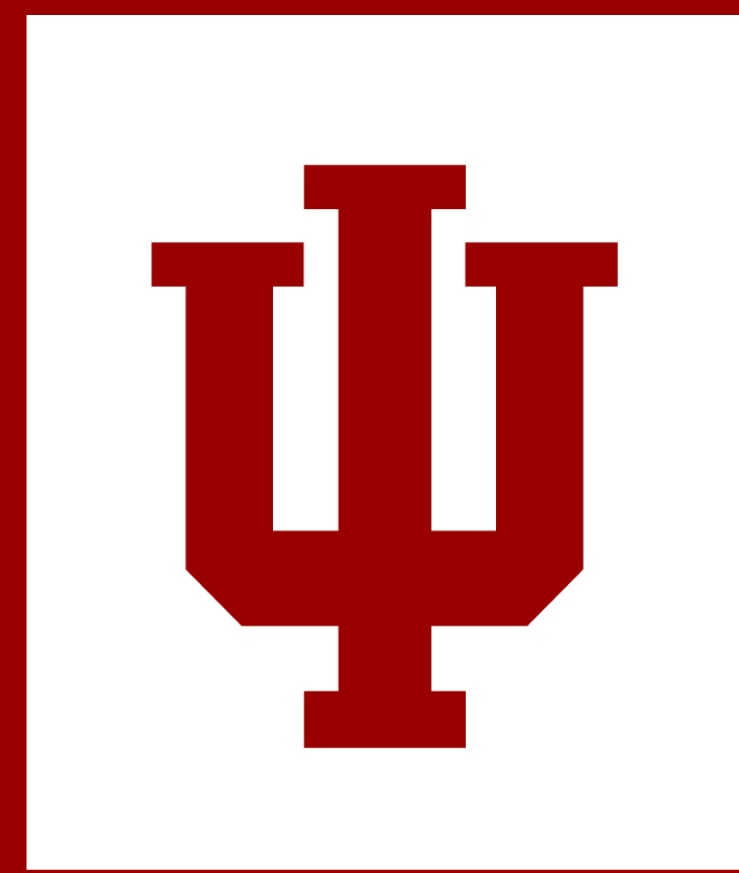




Amyloid- β Induces MR1 Expression and MAIT Cell Activation While an MR1-deficiency Disrupts Microglial Phagocytosis and Signaling

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Abstract

Background: Increasing evidence supports a pivotal role for the innate immune system in Alzheimer's disease (AD). Our previous work showed that the innate-like T cell subpopulation, mucosal-associated invariant T (MAIT) cells, are increased and activated in 5XFAD mice. Furthermore, in MAIT cell-deficient 5XFAD mice (i.e., MR1-knockout), plaque pathology development is reduced, along with fewer dystrophic neurites, and improved cognition in a sex-dependent manner. However, the impact of MR1 expression on amyloid- β (A β) microglial phagocytosis remains unclear.

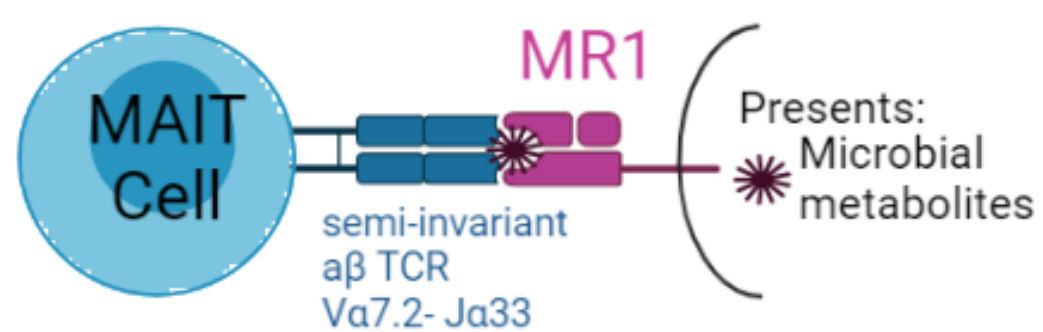
Methods: The murine BV-2 microglial cell line was first used in an *in vitro* assay to assess the impact of A β on microglial antigen presentation to MAIT cells. *In vivo*, Methoxy-X04 treatment followed by flow cytometry was used to analyze the phagocytic capacity of brain microglia from various ages of MR1+ or MR1-deficient (MR1 KO) 5XFAD mice. Immunofluorescent microscopic analysis of microglia in the hippocampus and cortex was performed with antibodies against microglial phagocytic and lysosomal markers, including: ST2, Trem2, CD68, TMEM106B, CD33, SHIP1, and CD47.

Results: The *in vitro* assays showed that A β induced elevated surface MR1 expression in the murine BV-2 microglial cell line, and consequently enhanced MR1-dependent MAIT cell activation. *In vivo*, we found that microglial phagocytosis of A β was significantly reduced in the CD11b⁺CD45^{low} population of 6-month-old 5XFAD/MR1 KO mice. The populations of microglia that lacked MR1 displayed distinct sex- and region-specific decreases in the lysosomal marker CD68, and a reduction in phagocytic markers, as well as in the disease-associated markers, Trem2 and ST2. Interestingly, there was also a decrease in levels of the "don't eat me" signaling molecule SHIP1 in MR1-deficient microglia.

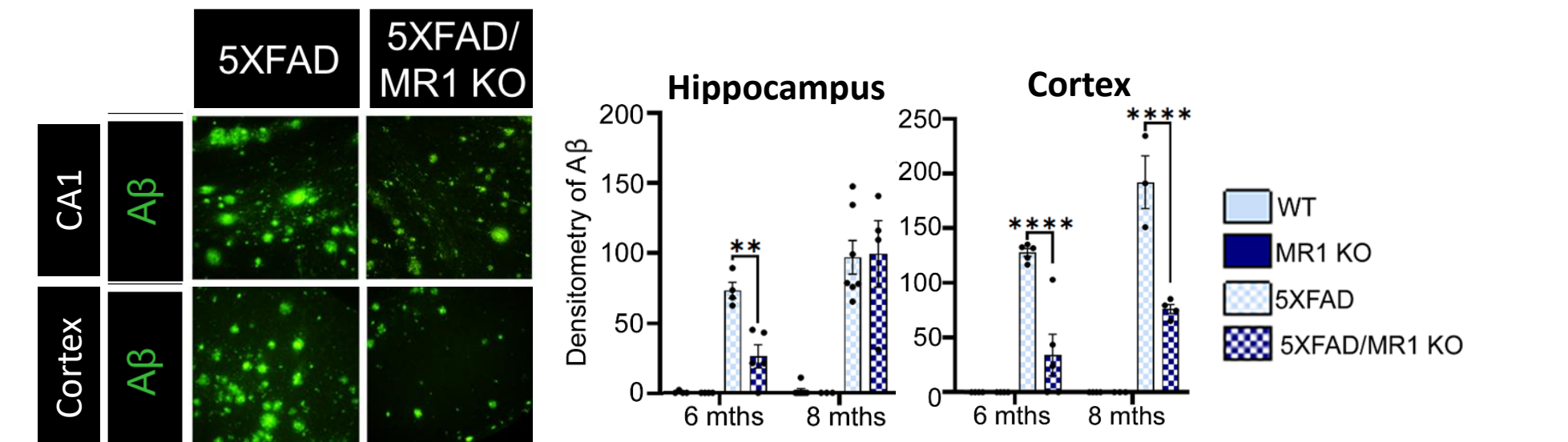
Conclusion: These results provide strong support for associations between A β and the functional expression of MR1, as well as in the MR1-dependent regulation of microglial responses in AD, offering new insights into the immune-related mechanisms driving AD pathology.

Introduction

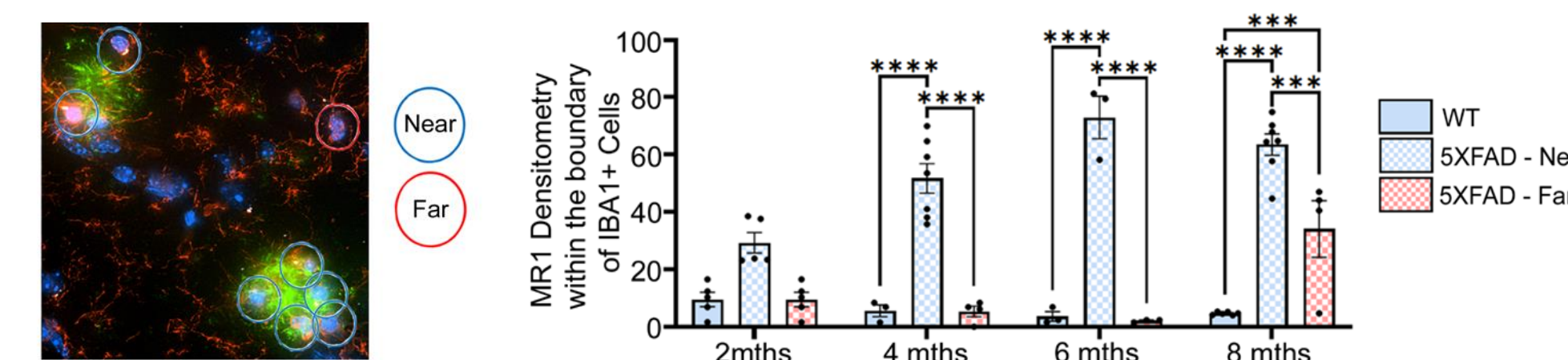
- Alzheimer's disease (AD) is a type of dementia that affects memory, processing, and behavior [1-3]
- The MR1/MAIT cell axis has become important in many CNS diseases [4-5]. More recently, we have found MAIT cells activated in the brains and livers of the 5XFAD mouse model of AD [6; 7].



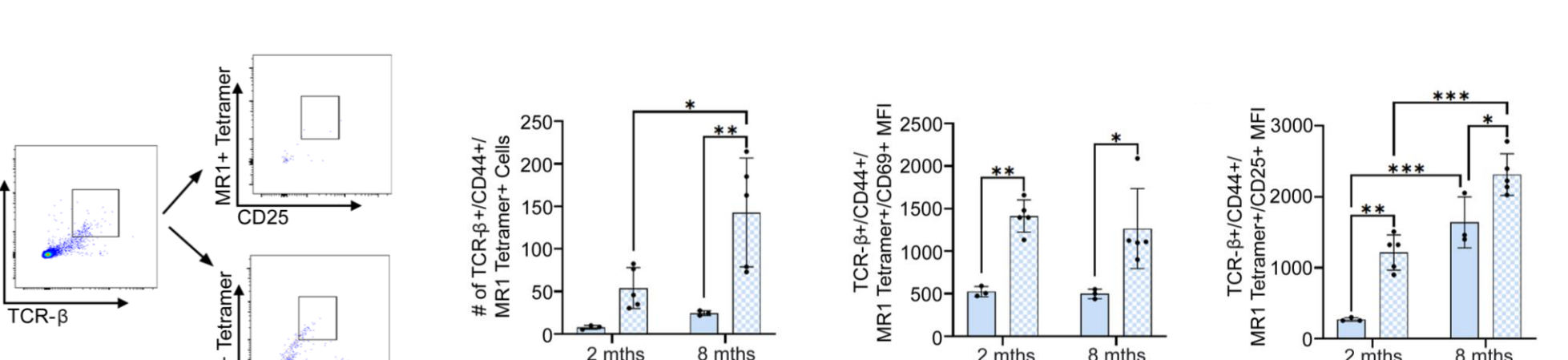
- Previously, our lab showed that the loss of the MR1/MAIT cell axis reduces amyloid beta (A β) plaques in 5XFAD mice [7].



- We have also shown that there are increased levels of MR1 within IBA1+ cells near A β compared to those far from A β [7].

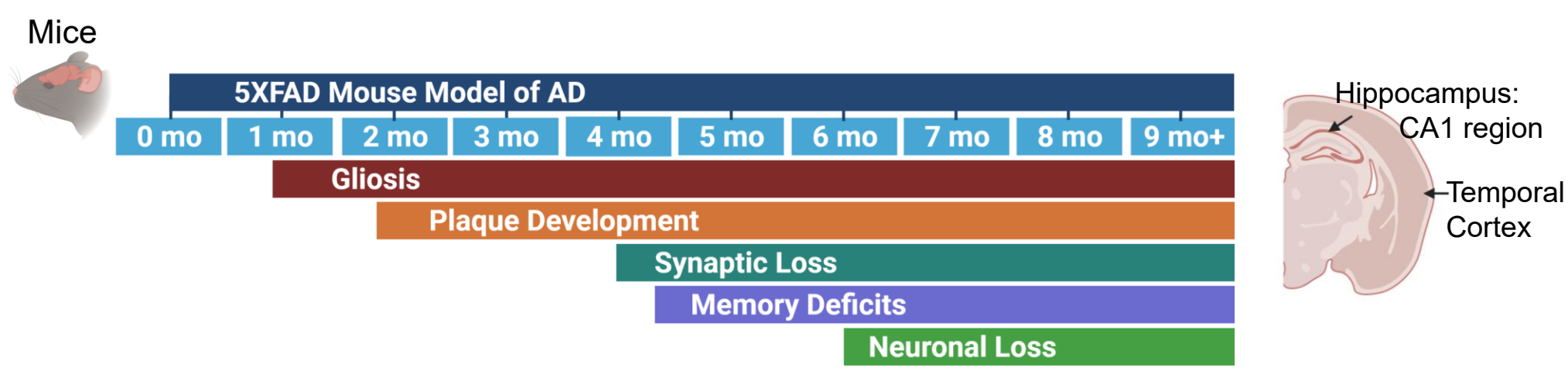


- MAIT cells are highly activated in 5XFAD mouse brains and livers



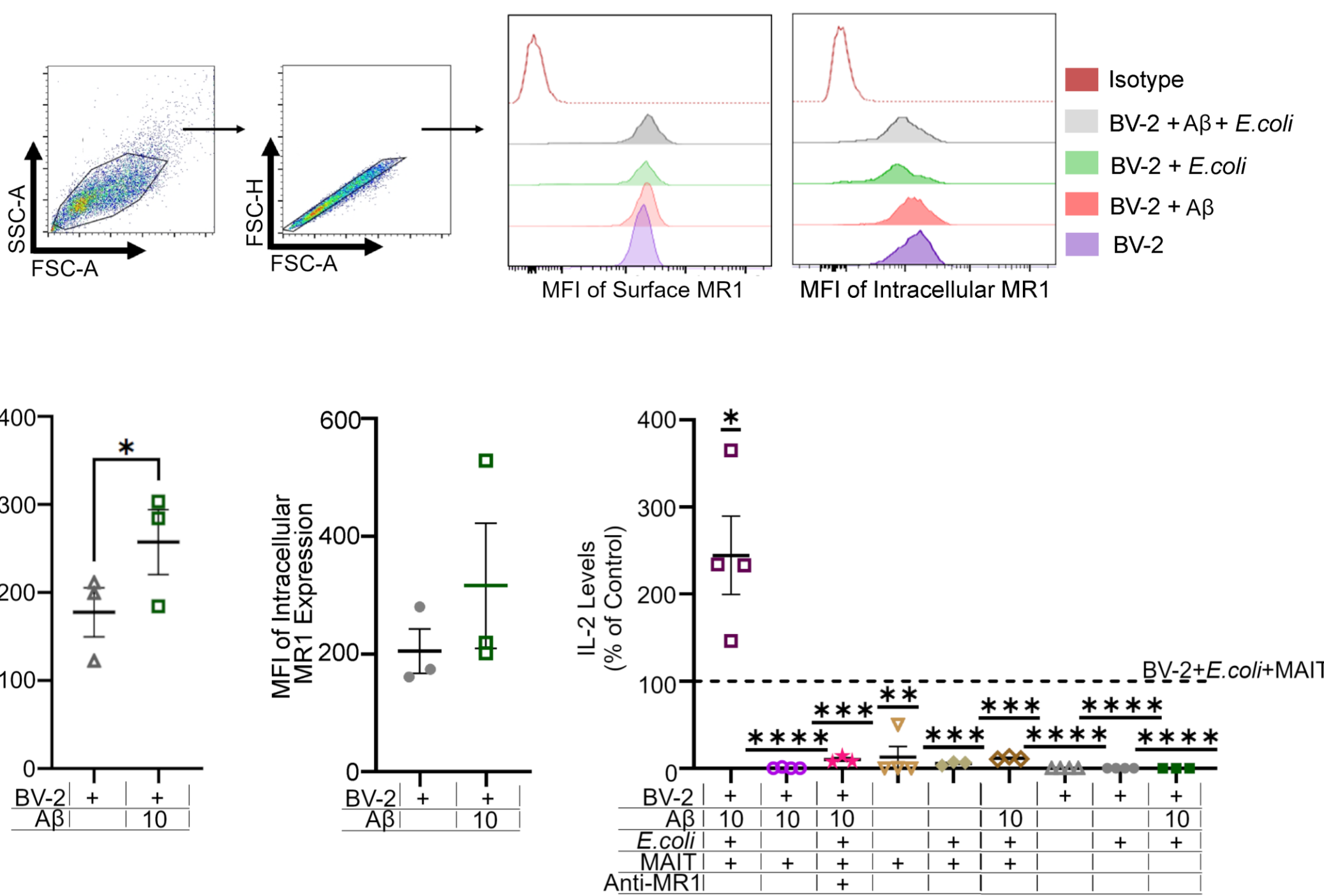
- Our *hypothesis* is that A β -induces MR1 expression on microglia which increases MAIT cell activation.

Materials & Methods

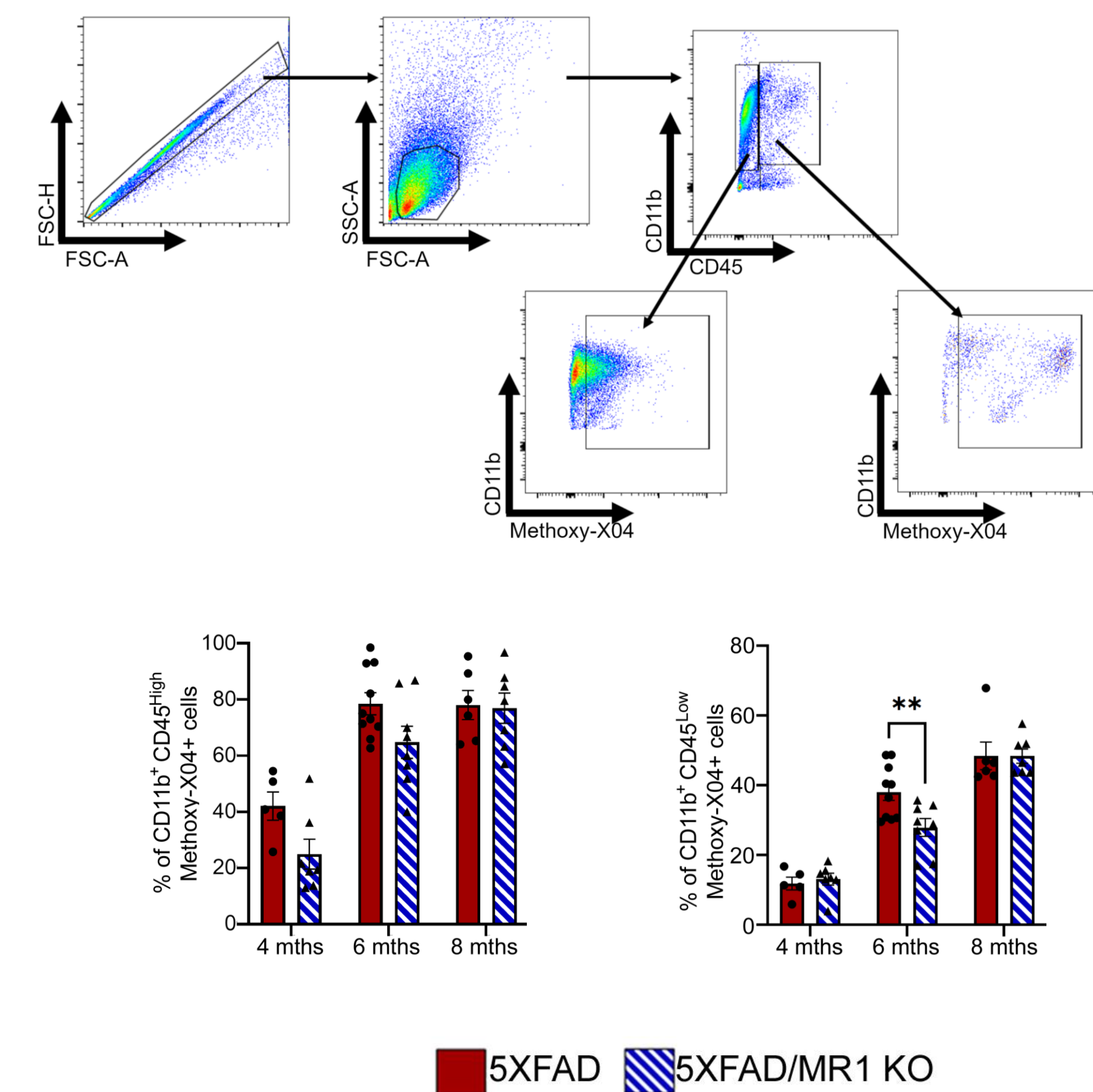


* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

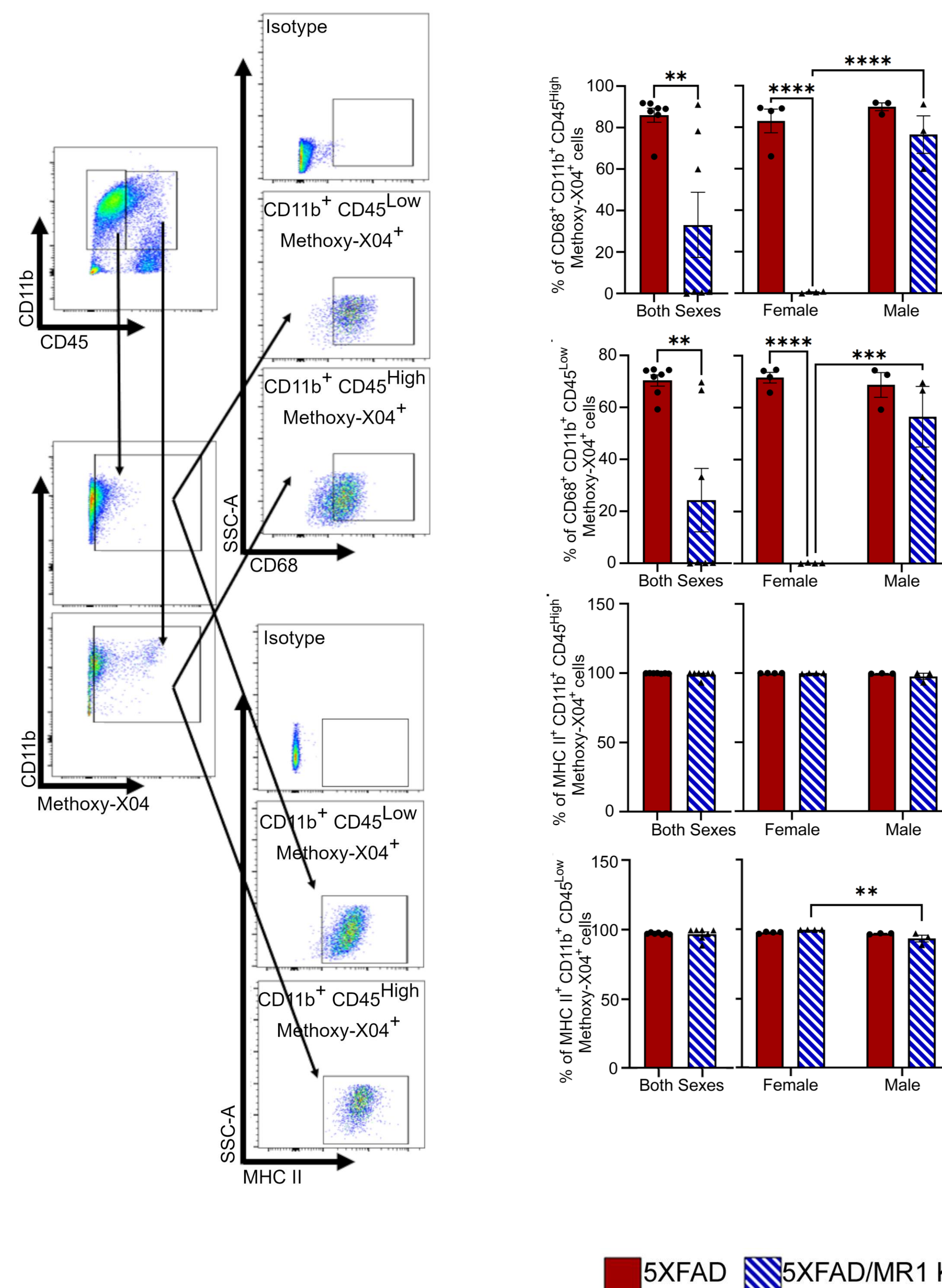
A β enhances microglial MR1 expression and MR1-dependent MAIT cell activation



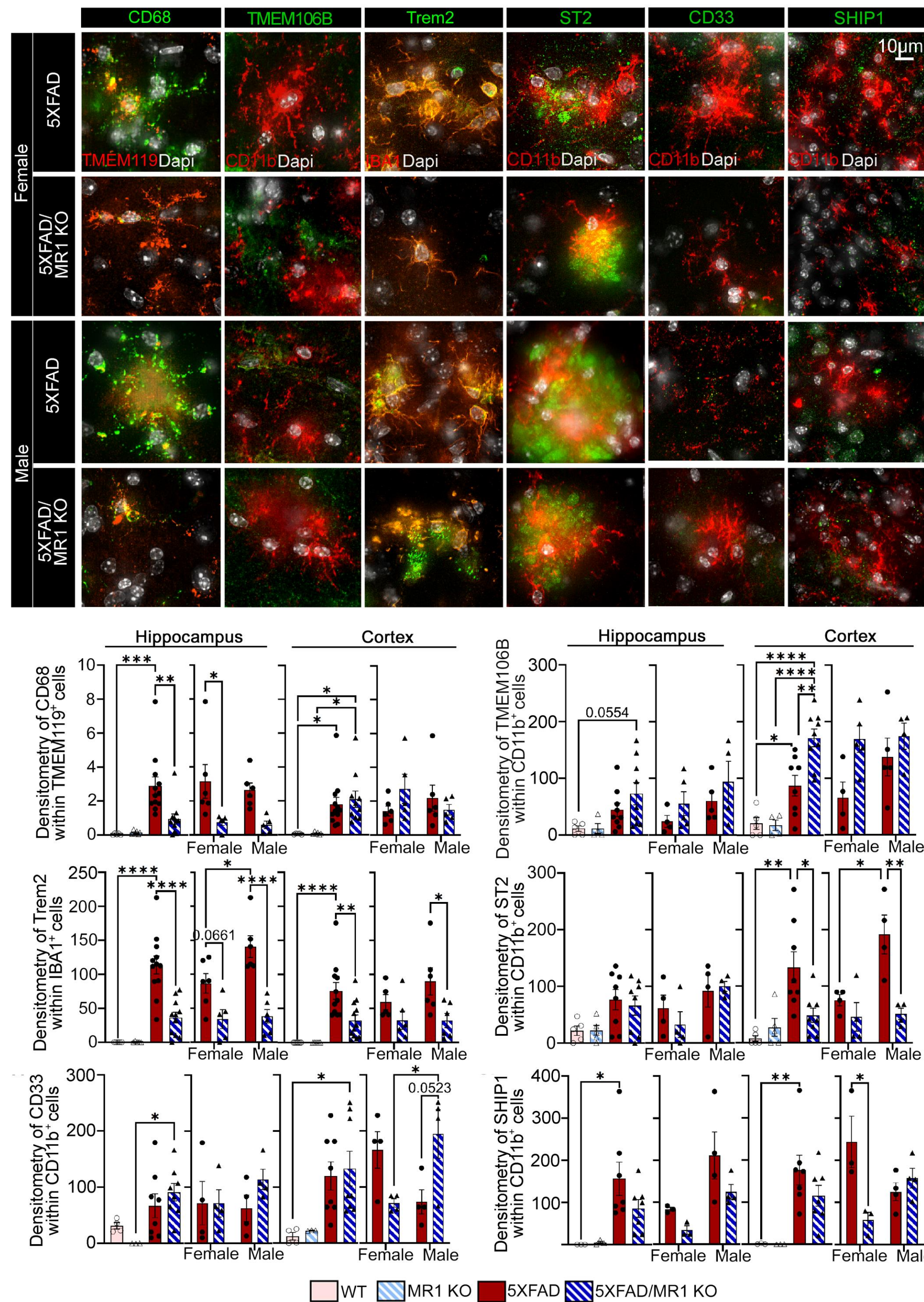
Reduced phagocytosis of A β in MR1-deficient microglia



Female 5XFAD mice that lack the MR1/MAIT cell axis have reduced CD68 expression



5XFAD/MR1 KO microglia have reduced Trem2 and ST2



Summary & Conclusions

- MR1 is increased on the surface of BV-2 microglial cells when exposed to A β , while intracellular expression remains unchanged.
- In vitro* MAIT cells are highly activated when co-cultured with BV-2 microglial cells, *E. coli*, and A β compared to just BV-2 microglial cells and *E. coli* alone.
- In vivo*, CD45^{low} microglia from 5XFAD/MR1 KO mice showed reduced phagocytosis at 6 months compared to CD45^{low} macrophages/microglia cells.
- Loss of MR1 resulted in reduced expression of the lysosomal marker CD68, particularly in female mice.
- MR1 deficiency was associated with lower levels of disease-associated microglial markers Trem2 and ST2.
- SHIP1 and CD33, phagocytic inhibitory molecules, remained unchanged between 5XFAD and 5XFAD/MR1 KO.
- Overall, the results of this study support a critical role for microglial MR1 in the response to A β , thereby reinforcing a detrimental role for MR1 in AD and providing additional insights into how MR1 contributes to A β pathology development.

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*Diagrams made with Biorender.com

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