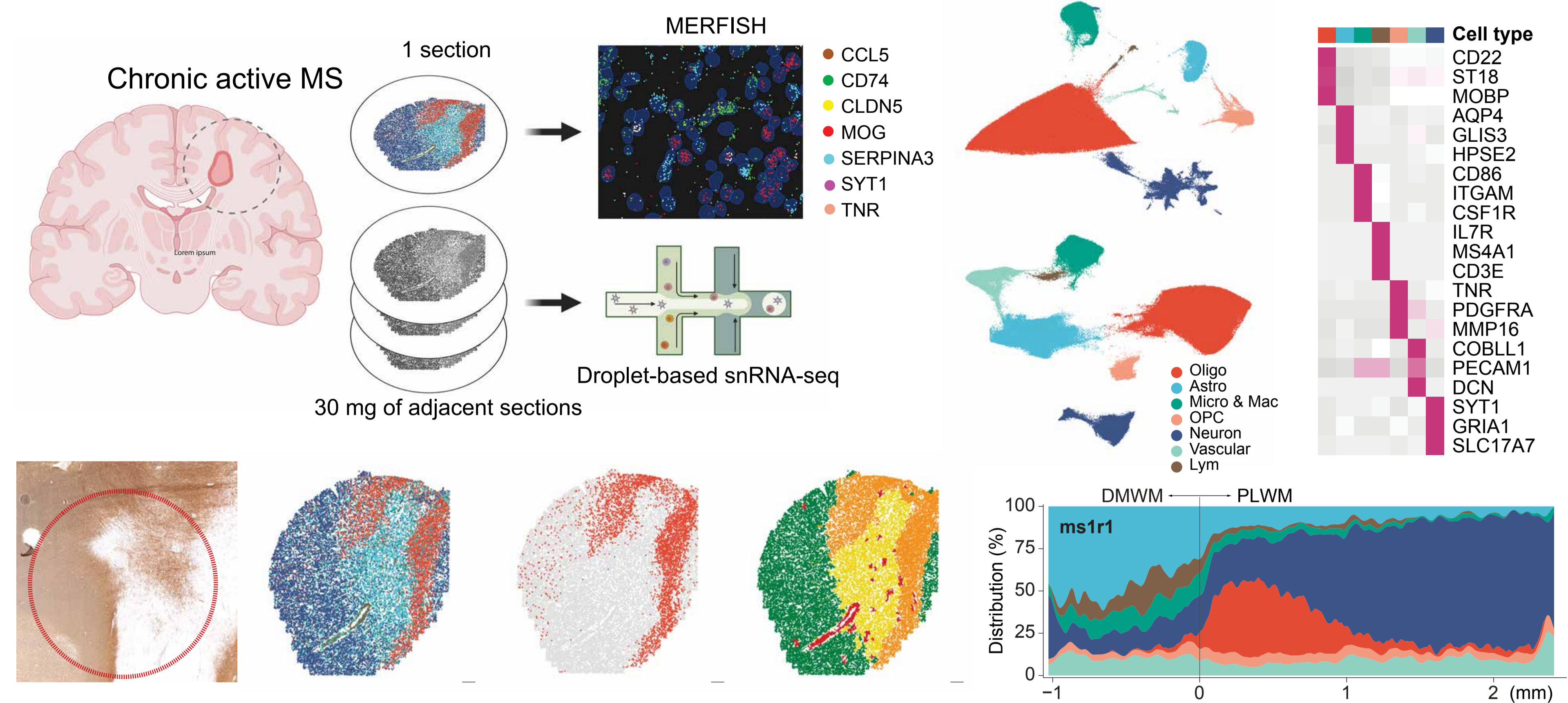


Single-cell spatial transcriptomic profiling defines a pathogenic inflammatory niche in chronic active multiple sclerosis lesions

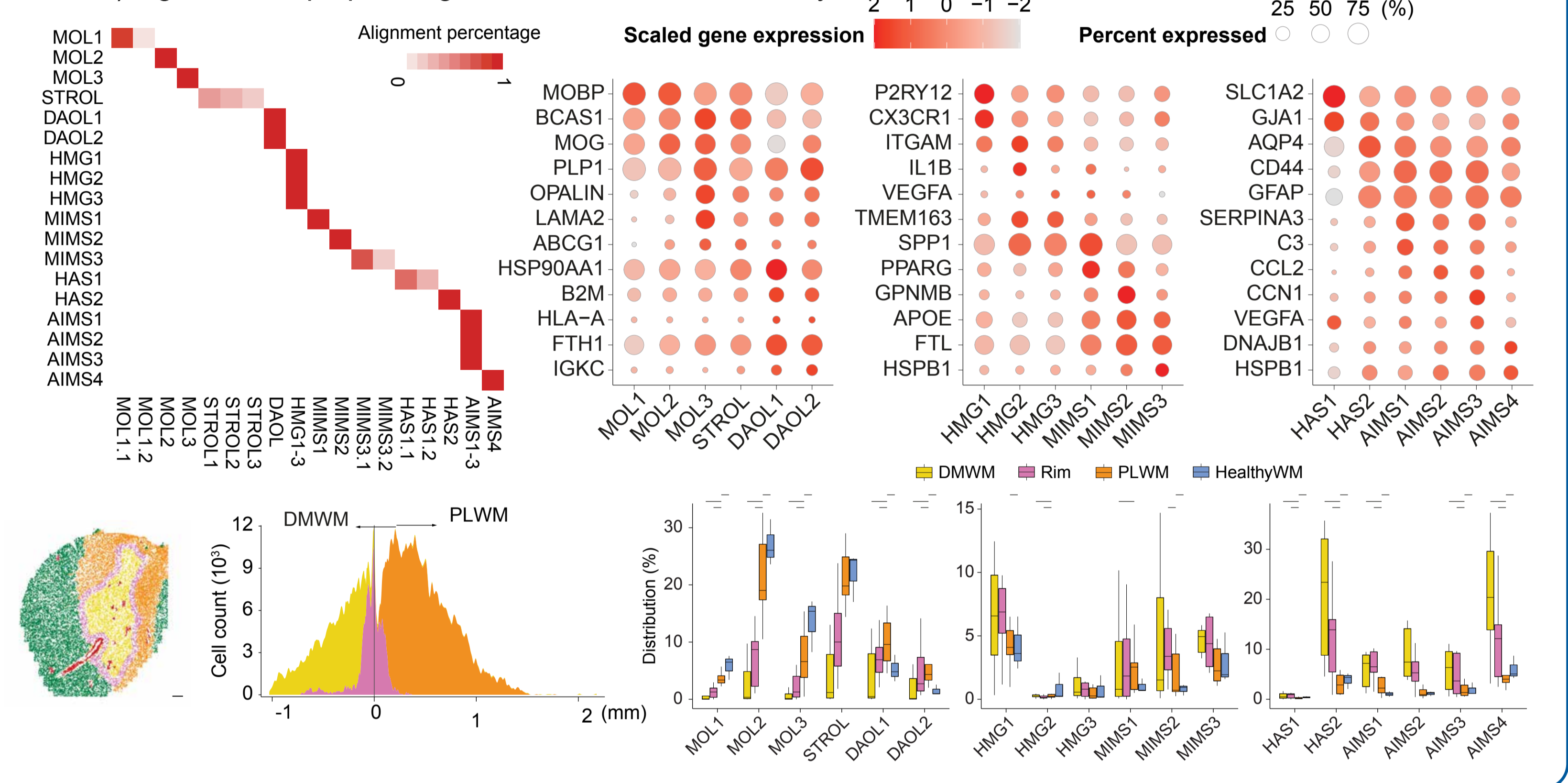
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Introduction

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system characterized by demyelination and neurodegeneration. Despite therapies that curb peripheral immunity, many patients continue to worsen, implicating persistent, compartmentalized inflammation within the brain. A hallmark of this process is the chronic active lesion: a demyelinated core encircled by a rim of chronically activated microglia that is thought to impede repair and sustain tissue damage. Prior transcriptomic studies have cataloged diverse reactive glial states, yet the specific cellular neighborhoods and immune–glial interactions that maintain this smoldering pathology remain unclear. Clarifying how T cells interface with microglia—and how lipid handling and interferon signaling shape microglial function—could reveal mechanisms that perpetuate inflammation and nominate metabolic axes as therapeutic entry points for progressive MS.

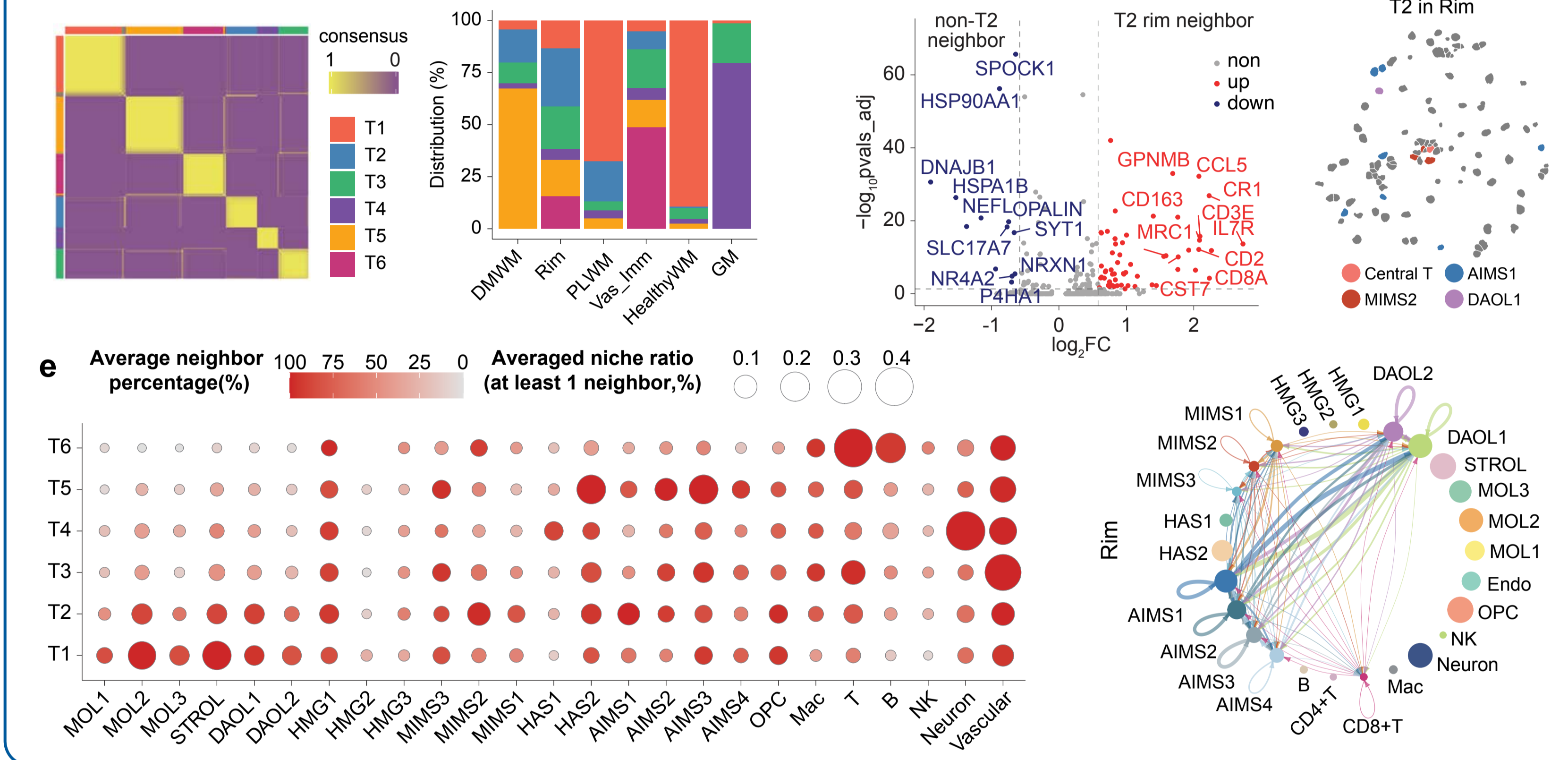


Sub-clustering and spatial mapping identify reactive glial cell states across distinct lesion domains
Cross-modal MERFISH–snRNA-seq mapping with SPATA2 gradients and BANKSY segmentation charts reactive glial states across DMWM > lesion rim > PLWM. The rim is enriched for MIMS2 (GPNMB+, lipid/IFN-responsive) microglia and inflammatory lipid-metabolism programs, whereas the demyelinated core shows higher MIMS3 (FTH+, stress/iron) signatures—pinpointing the rim as the inflammatory hub.



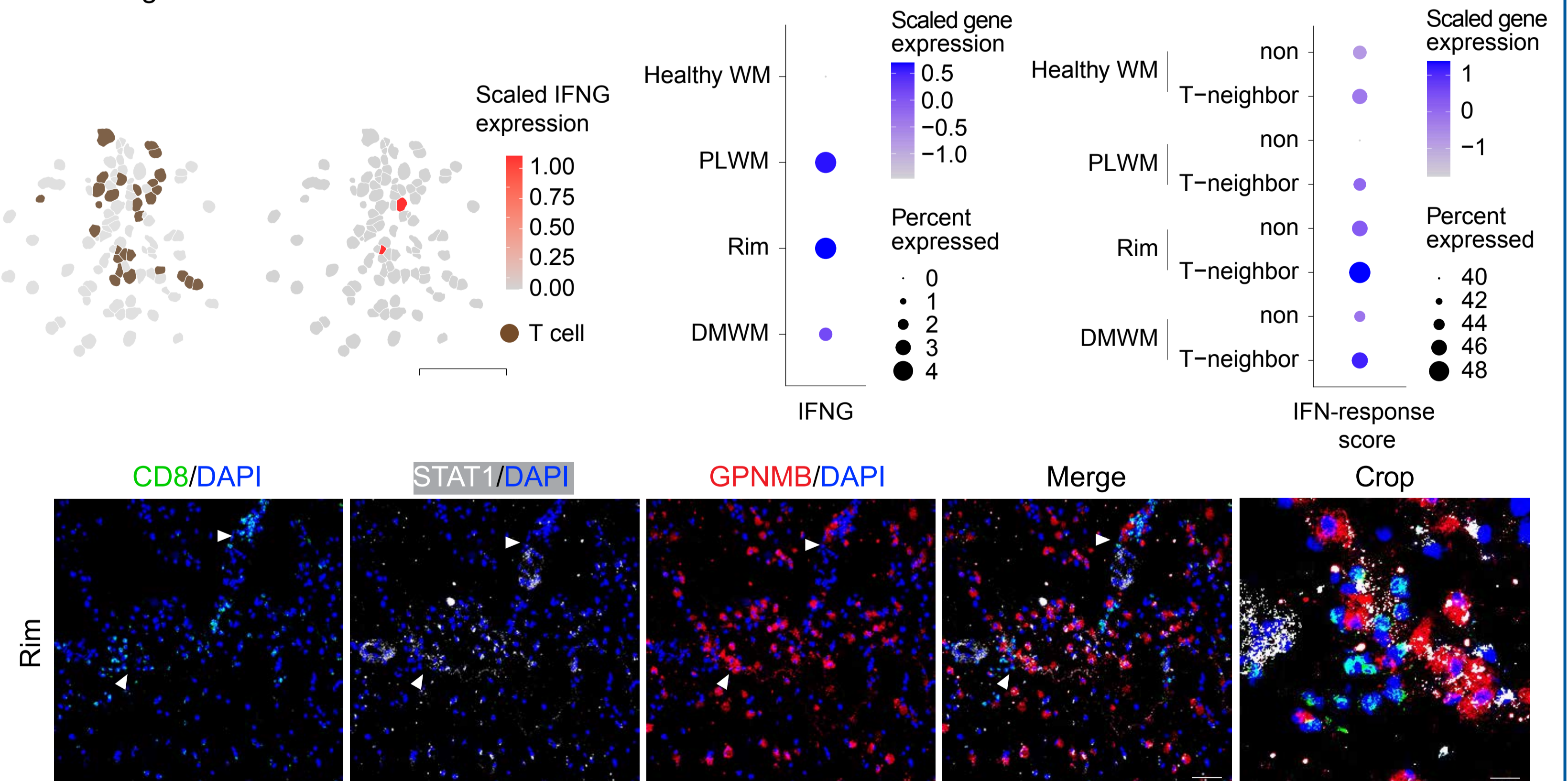
Interactions between peripheral immune cells and reactive glia shape distinct lesion domains

Peripheral immune infiltration organizes into distinct T-cell niches across lesion domains, with CD8+ T cells enriched at the lesion rim. The rim-localized T2 niche sits next to GPNMB+ (MIMS2) microglia, and ligand–receptor analysis predicts the strongest bidirectional signaling between CD8+ T cells and MIMS2 in rim/DMWM, highlighting a pathogenic immune–glial hub.



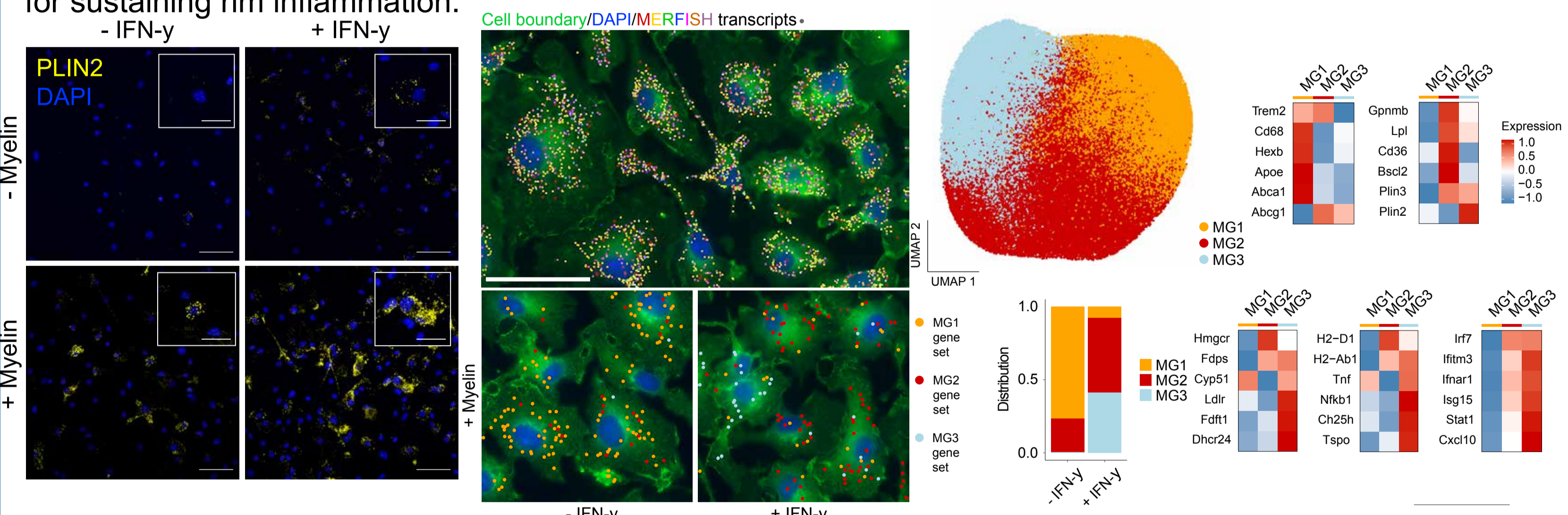
CD8+ T cells associate with GPNMB+ STAT1+ microglia at the chronic active lesion rim

Protein co-detection and spatial quantification show the lesion rim is enriched for MIMS2 (CD68+ GPNMB+) microglia and CD8+ T cells that lie in close proximity. CD8+ T cells at the rim express IFNG, nearby cells upregulate IFN-response genes, and GPNMB+ microglia adjacent to CD8+ T cells exhibit increased STAT1, indicating an interferon-linked CD8–microglia interaction that defines the chronic active rim.



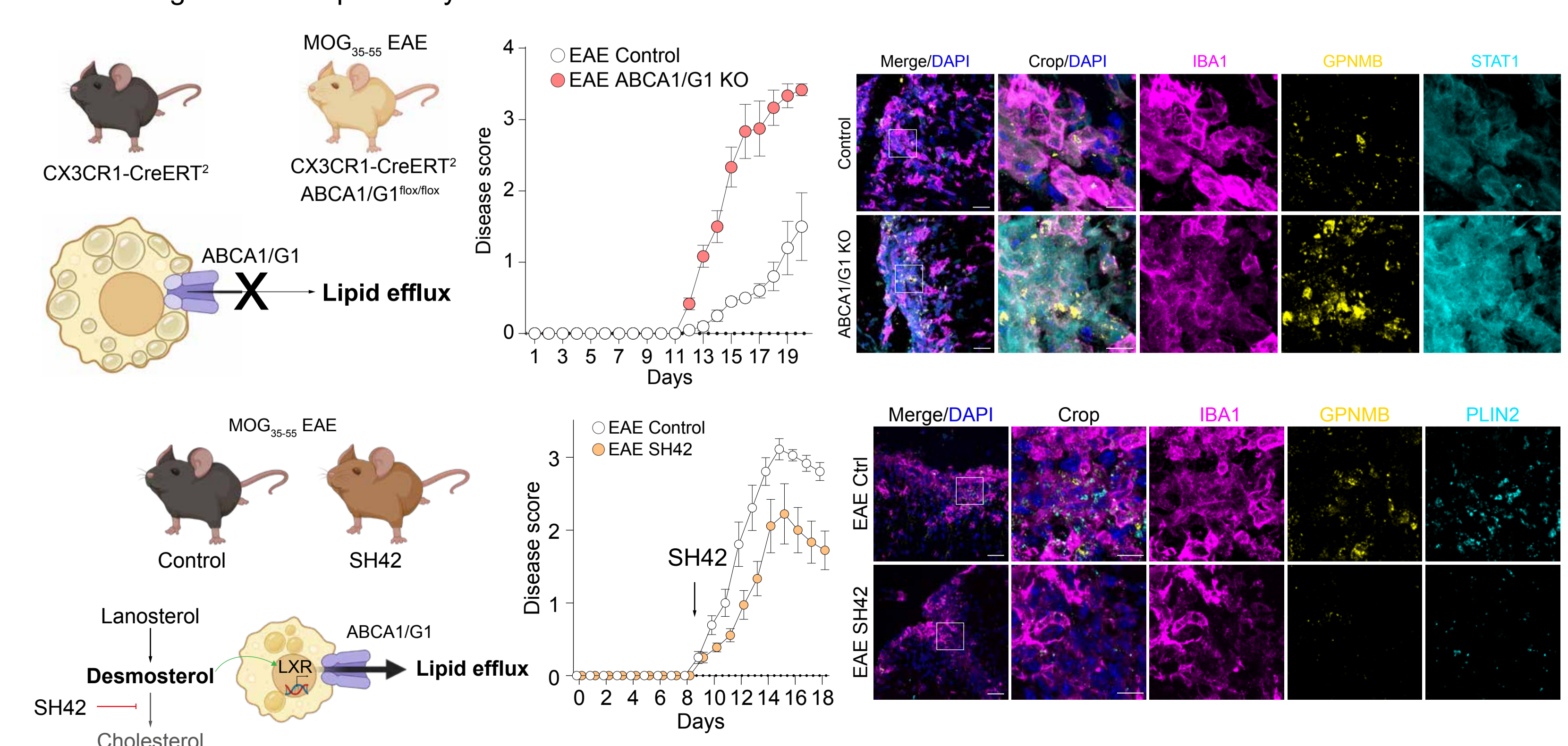
IFN-γ impairs microglial lipid metabolism after myelin phagocytosis

In primary microglia exposed to myelin, IFN-γ drives a foamy, inflammatory state—PLIN2↑/STAT1↑ with repression of cholesterol-efflux machinery (ABCA1/ABCG1↓)—and significantly reduces tracer cholesterol export. These data link T-cell-derived interferon to impaired microglial lipid handling as a proximal mechanism for sustaining rim inflammation.



Microglial Cholesterol Efflux Governs Rim Pathogenicity: Loss Worsens, Activation Ameliorates

Genetic disruption of Abca1/Abcg1 in microglia worsens EAE with increased lesion burden, CD8+ T-cell infiltration, and a foamy GPNMB+/PLIN2+/STAT1+ microglial state, whereas pharmacologic activation of the efflux/LXR axis (e.g., SH42) restores Abca1, dampens these signatures, reduces lesions and clinical scores. In short, blocking efflux fuels the rim, and boosting efflux therapeutically reverses it.



conclusions:

- Chronic active MS lesions harbor a rim niche where CD8+ T cells about GPNMB+, IFN-responsive, lipid-laden microglia.
- This immune–glial neighborhood shows the strongest bidirectional signaling at the rim, marking it as the inflammatory hub.
- IFN-γ from T cells impairs microglial cholesterol efflux (ABCA1/ABCG1↓), driving a foamy, pro-inflammatory microglial state.
- Disrupting microglial Abca1/Abcg1 worsens EAE, increasing lesion burden, CD8+ infiltration, and GPNMB+/PLIN2+/STAT1+ signatures.
- Pharmacologically activating sterol efflux/LXR (e.g., SH42) restores Abca1, reduces foamy microglia, and ameliorates disease.
- Spatial single-cell mapping resolves lesion domains (DMWM → rim → PLWM) and locates reactive glial states with high precision.