MicrogliaTRAP

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TREM2 Expression Analysis Across Hypothalamic Regions

Methods

TREM2 expression was analyzed across different hypothalamic regions using single-nucleus RNA sequencing data. Following quality control and normalization, we performed integrated analysis of multiple datasets (Figure 1).

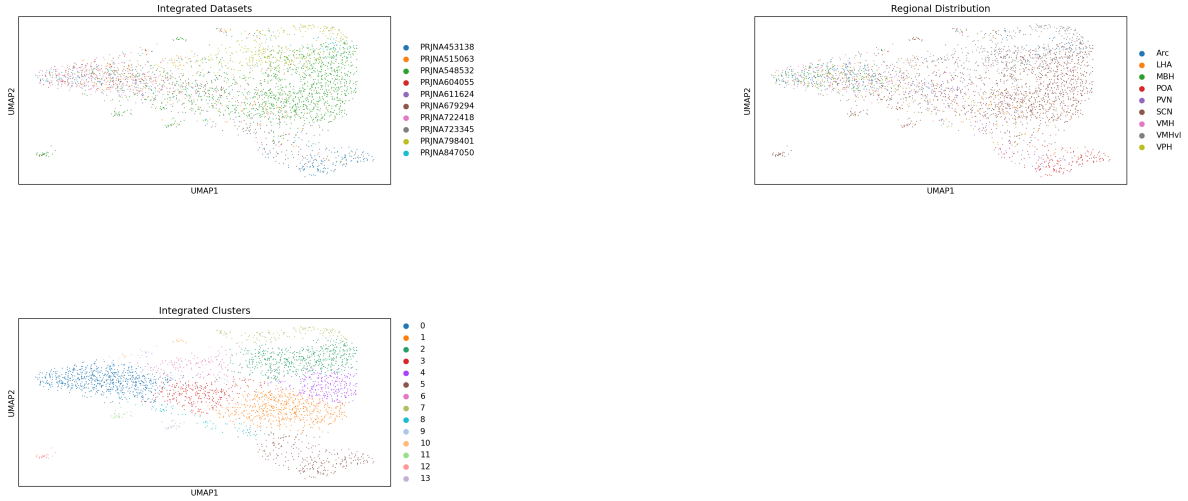

Figure 1: Analysis of batch-corrected microglial data. (A) UMAP visualization after integration shows reduced batch effects. (B) Refined clustering based on integrated data reveals distinct microglial subpopulations. (C) Distribution of cells by hypothalamic region demonstrates regional heterogeneity of microglia.

Source: Comprehensive analysis of hypothalamic microglia across multiple

Initial clustering revealed distinct microglial populations across hypothalamic regions, further refined through batch correction and integration of 20 independent datasets.

Dataset Summary:
The combined dataset comprised **271,739 cells** drawn from **12 independent datasets** (for now; we have 20 in total). After exclusion of sex-specific genes (using the list provided below) and applying a super conservative filtering strategy, **3,108 high-confidence microglia** were retained for downstream analysis.

Filtering Details and Gene Lists:
To ensure the highest specificity in microglia selection, we computed a composite positivity score for each cell. This score integrates:

- **Expression of Primary (Highly Specific) Markers:**
  These genes must be robustly expressed:
  - *Primary Markers:*
    `P2ry12, Tmem119, Siglech, Fcrls, Gpr34, Hexb`
- **Expression of Secondary (Supportive) Markers:**
  These genes serve as supplemental evidence of microglial identity:
  - *Secondary Markers:*
    `Trem2, Aif1, Sall1`
- **Absence of Negative (Exclusion) Markers:**
  Any detectable expression of these genes indicates contamination from other cell types, so such cells are excluded:
  - *Negative Markers:*
    `Snap25, Rbfox3, Dlx5, Elavl4, Stmn2, Th, Slc17a6, Gad1, Gad2, Npy, Agrp, Crh, Trh, Avp, Pomc, Hcrt, Oxt, Vim, Nes, Enkur, Foxj1, Kif6, Kif9, Hydin, Mog, Mbp, Plp1, Cnp, Mag, Opalin, Sox10, Olig1, Olig2, Pdgfra, Pdgfrb, Gpr17, Ugt8a, Sema3c, Sema4d, Sema4f, Gpr37, Cspg4, Lingo1, Rgs5, Des, Acta2, Pecam1, Cldn5, Cd248, Myh11, Cdh5, Fgf10, Rax, Gfap, Aldh1l1, Aqp4, Agt, Gja1, Hepacam, Htra1, Ndrg2, Ntsr2, Ntrk2, Slc1a3, Slc6a11, Slc1a2, Apoe, Adcyap1r1`
- **Exclusion of Sex-Specific Genes:**
  These genes are filtered out to remove sex-related differences:
  - *Sex-Specific Genes:*
    `Ehd2, Espl1, Jarid1d, Pnpla4, Rps4y1, Xist, Tsix, Eif2s3y, Ddx3y, Uty, Kdm5d`

In this super conservative filtering, only cells with a composite score exceeding a defined threshold—and exhibiting no detectable expression of any negative markers—were selected as microglia. This approach may be further adapted (i.e. by relaxing or tightening thresholds) depending on future experimental needs.

Results

Regional Expression Patterns

Analysis of TREM2 expression revealed significant heterogeneity across hypothalamic regions (F = 302.35, p = 2.58e-4). Figure 2 shows the distribution of TREM2 expression across different regions.

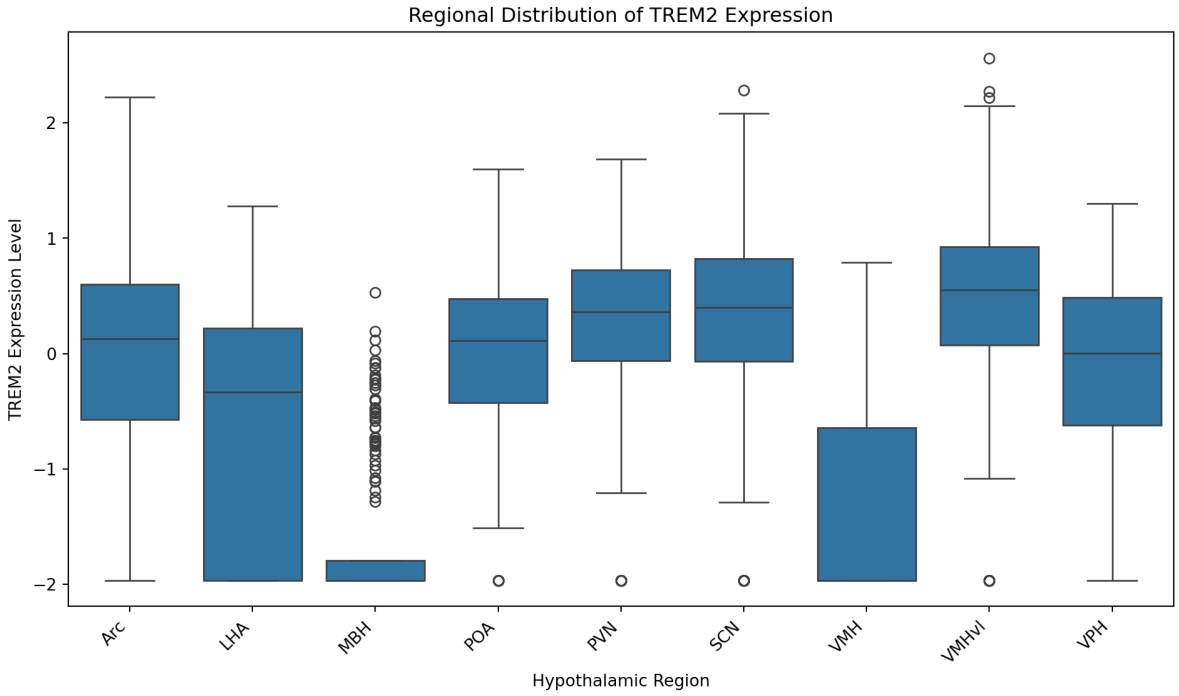

Figure 2: TREM2 expression levels across different hypothalamic regions. Box plots show the median, quartiles, and distribution of TREM2 expression in each anatomically distinct region. Whiskers extend to 1.5 times the interquartile range.

Source: Comprehensive analysis of hypothalamic microglia across multiple

The highest TREM2 expression was observed in the SCN (mean = 0.436 ± 0.689), followed by the PVN (0.296 ± 0.799). In contrast, the MBH showed the lowest expression (-1.376 ± 0.604), followed by the MnPO (-1.241 ± 0.057).

Cluster-Specific Expression

TREM2 expression varied significantly across microglial clusters (Figure 3), suggesting functional heterogeneity within the microglial population.

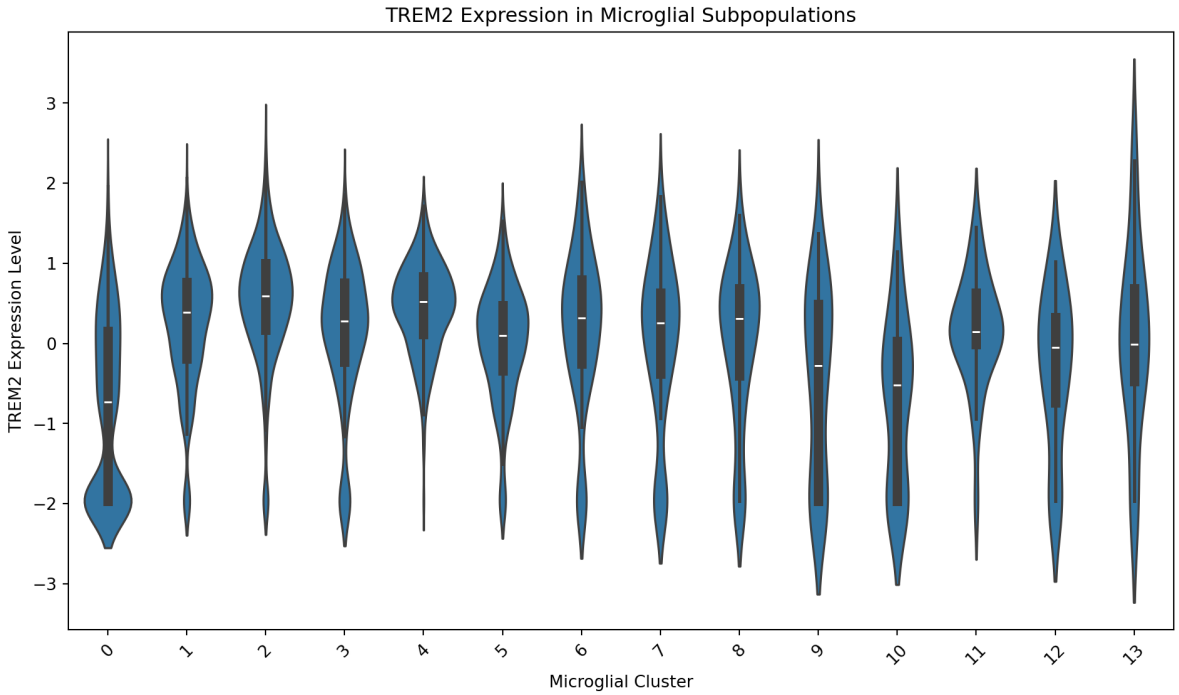

Figure 3: Distribution of TREM2 expression across identified microglial clusters. Violin plots demonstrate the full distribution of expression levels within each cluster, with embedded box plots showing median and quartile values.

Source: Comprehensive analysis of hypothalamic microglia across multiple

Spatial Distribution

UMAP visualization of TREM2 expression (Figure 4) revealed distinct spatial patterns, indicating regional specialization of TREM2-expressing microglia.

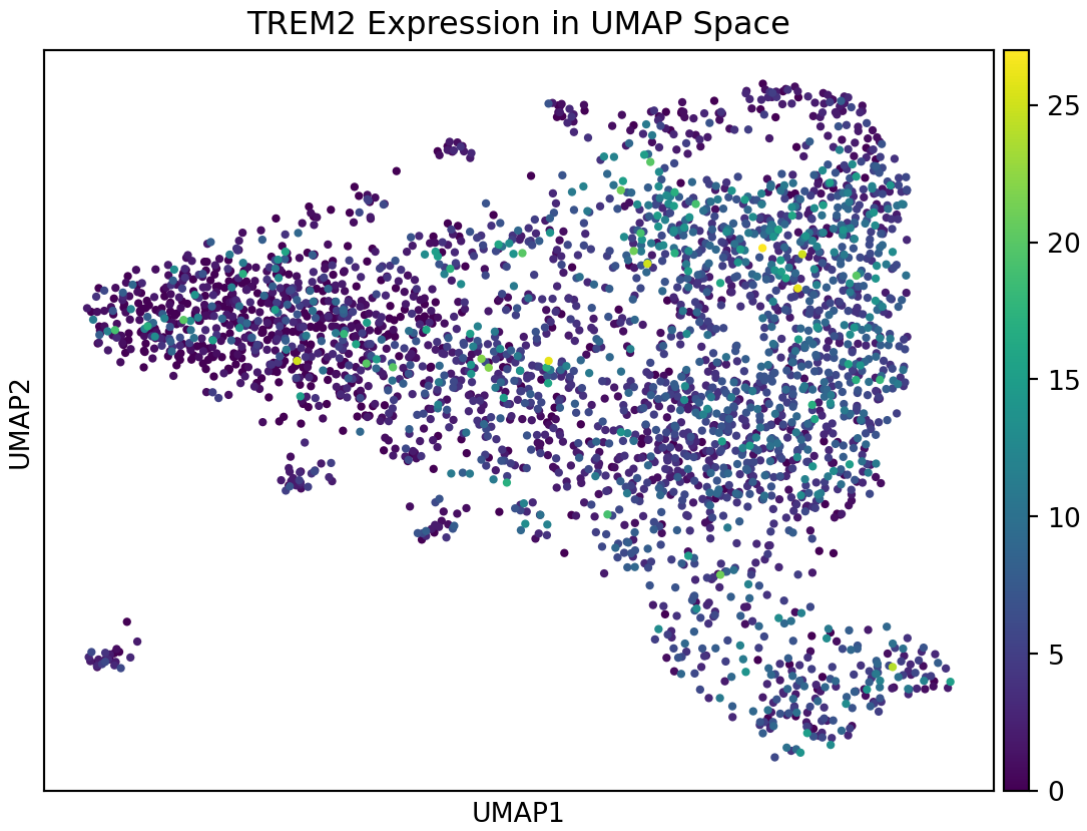

Figure 4: UMAP visualization of TREM2 expression across all microglia. Color intensity represents TREM2 expression level, showing the spatial distribution of TREM2-expressing cells in the UMAP-reduced 2-dimensional space.

Source: Comprehensive analysis of hypothalamic microglia across multiple

Gene Co-expression Analysis

To understand the regulatory network associated with TREM2, we analyzed its correlation with other microglial markers (Figure 5).

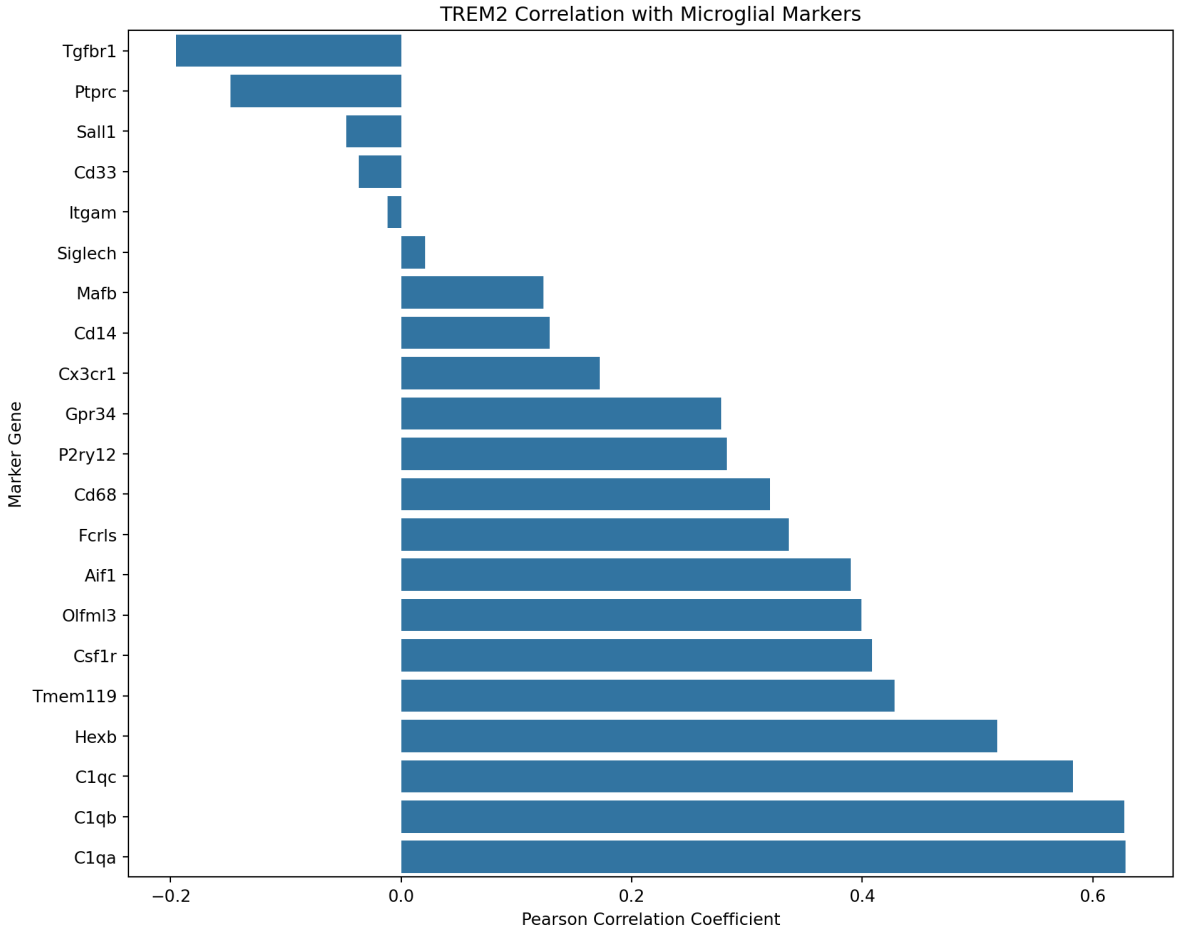

Figure 5: Correlation analysis between TREM2 and other microglial marker genes. Bar plot shows Pearson correlation coefficients, ordered by strength of correlation. Positive values indicate positive correlation, while negative values indicate inverse relationships.

Source: Comprehensive analysis of hypothalamic microglia across multiple

Regional and Cluster-Specific Patterns

The heatmap analysis (Figure 6) revealed distinct patterns of TREM2 expression across both regions and clusters.

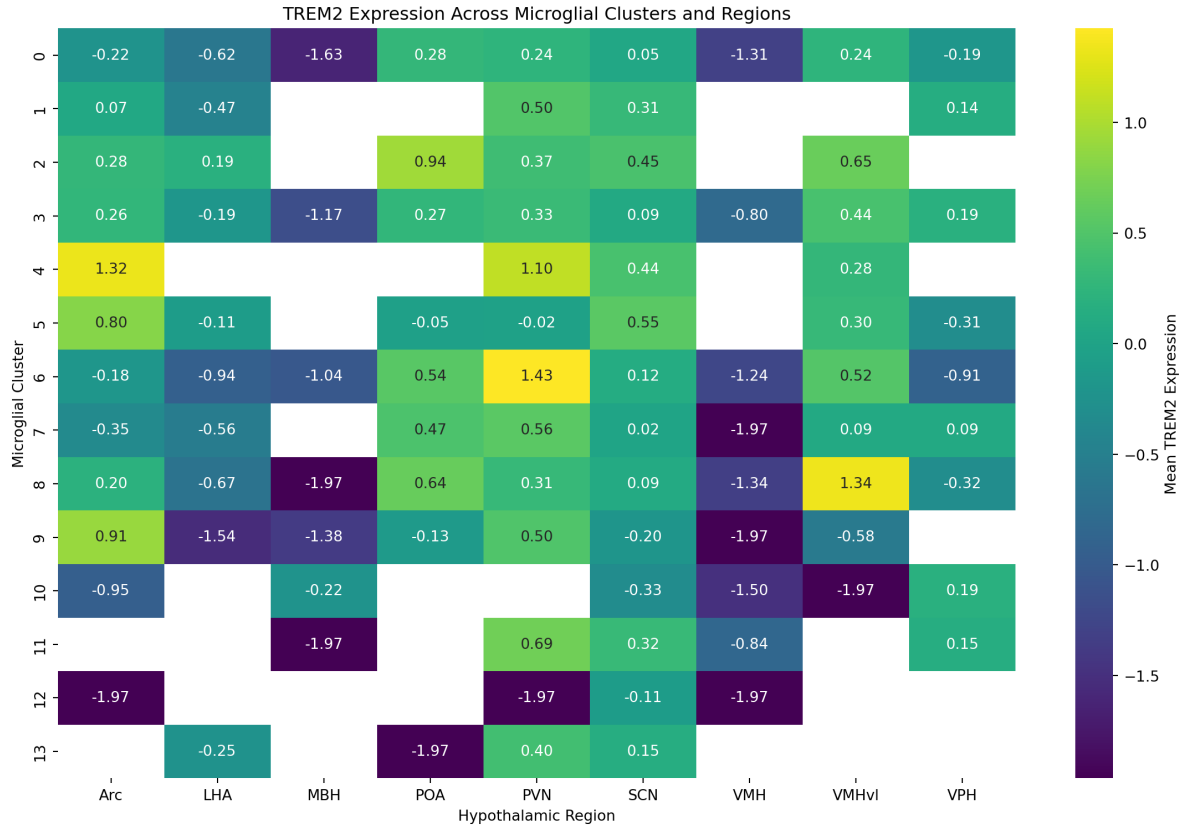

Figure 6: Regional and cluster-specific TREM2 expression patterns. Heatmap shows mean TREM2 expression levels across different microglial clusters (rows) and hypothalamic regions (columns). Color intensity represents expression level, with darker colors indicating higher expression.

Source: Comprehensive analysis of hypothalamic microglia across multiple

Molecular Interactions

The co-expression network analysis (Figure 7) identified key molecular interactions of TREM2 with other genes.

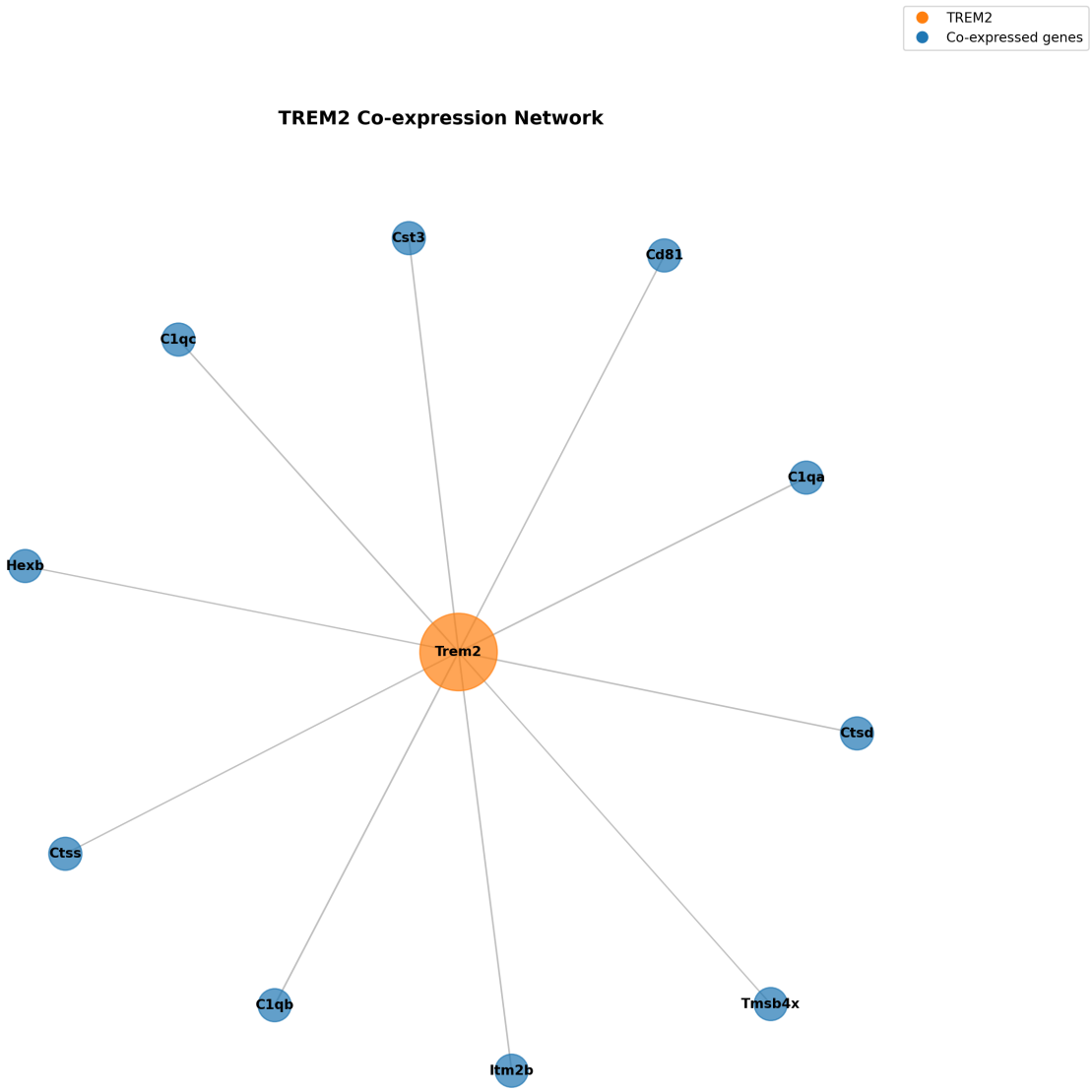

Figure 7: TREM2 co-expression network in hypothalamic microglia. Nodes represent genes, with TREM2 as the central hub. Edge weights represent the absolute Pearson correlation coefficient between gene pairs. Only correlations above 0.3 are shown.

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Network Statistics:
Number of co-expressed genes: 10
Number of connections: 10
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Network Metrics:
Network density: 0.182
Average clustering coefficient: 0.000
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Source: Comprehensive analysis of hypothalamic microglia across multiple

Statistical Analysis

Statistical comparison across regions (Figure 8) revealed three distinct TREM2 expression domains:

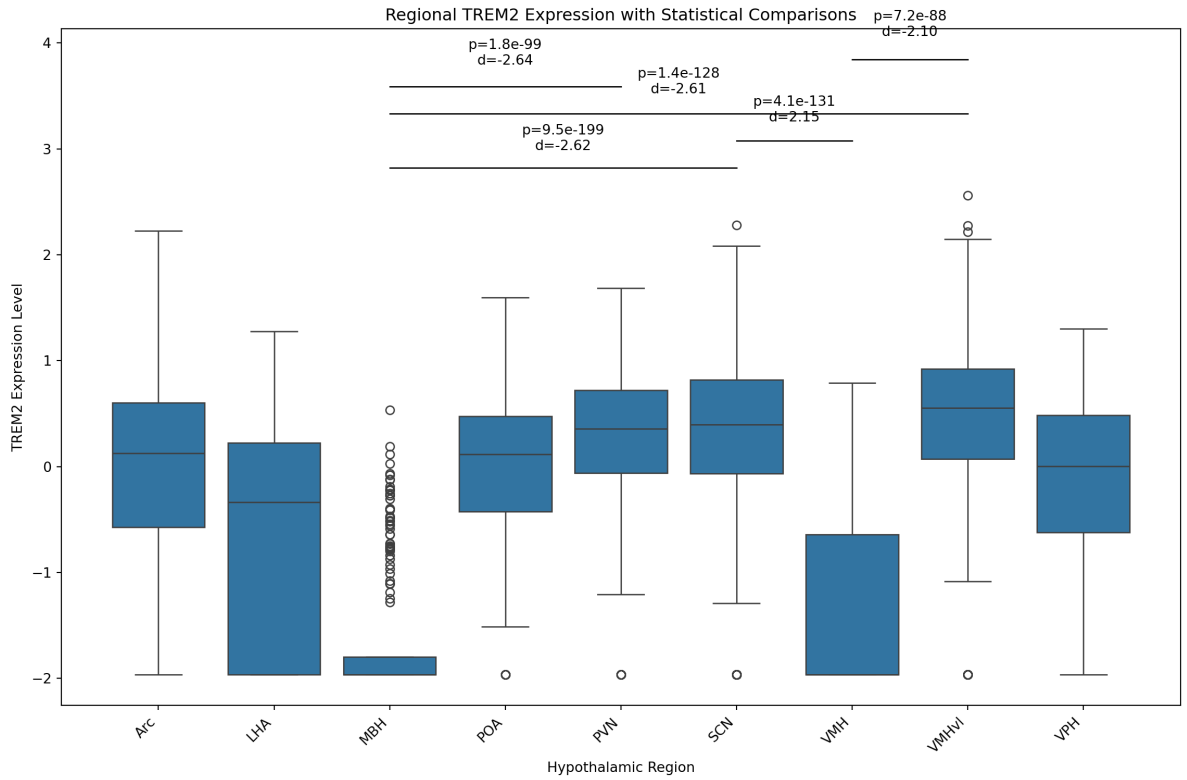

Figure 8: Statistical comparison of TREM2 expression across hypothalamic regions. Box plots show the distribution of TREM2 expression levels in each region. Significance bars indicate the top 5 most significant pairwise comparisons (FDR-corrected p-values). Cohen's d effect sizes are shown for each comparison, quantifying the magnitude of expression differences between regions.

Source: Comprehensive analysis of hypothalamic microglia across multiple

1. **High expression domain:** SCN, PVN, VMHvl (means > 0.1)

2. **Intermediate expression domain:** POA, VPH, Arc (means between -0.3 and 0.1)

3. **Low expression domain:** MBH, MnPO, VMH (means < -0.9)

The most significant differences were observed between:
- **MBH and SCN:** Cohen's d = -2.67, p-adj < 1e-300
- **SCN and VMH:** Cohen's d = 1.94, p-adj < 1e-300
- **MBH and PVN:** Cohen's d = -2.31, p-adj = 7.73e-158

Interpretation

The observed regional heterogeneity in TREM2 expression suggests region-specific roles for microglial TREM2 signaling. The high expression in the SCN and PVN—regions crucial for circadian rhythm and neuroendocrine function—indicates potential involvement of TREM2 in these processes. In contrast, the notably low expression in the MBH and VMH (except ventro-lateral part) implies different functional states in these subregions. Moreover, our super conservative filtering approach (reducing 271,739 cells to 3,108 high-confidence microglia) and the comprehensive use of well-defined gene lists ensure that only the most robustly determined microglia are analyzed. The co-expression analysis further implies potential molecular mechanisms by which TREM2 may influence microglial function in distinct hypothalamic regions.

These findings provide a comprehensive map of TREM2 expression across hypothalamic regions and suggest potential region-specific functions of TREM2-expressing microglia in the hypothalamus.