

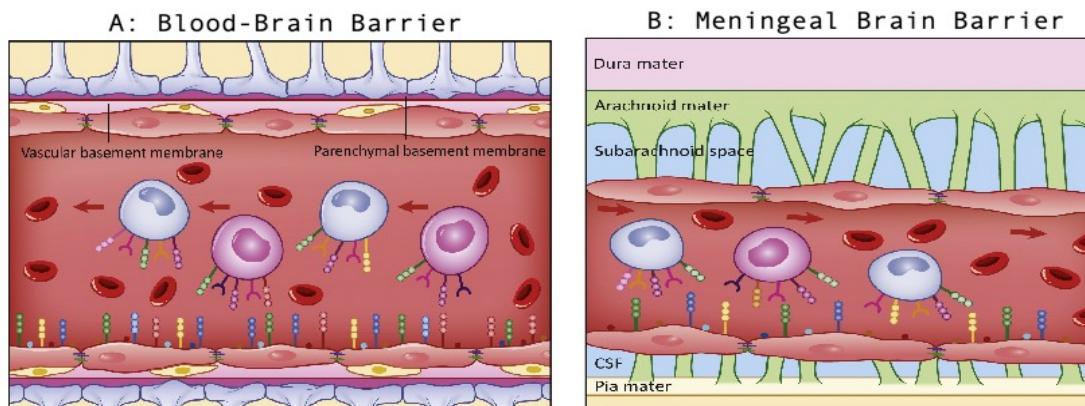
# Characterization of the transcriptional response of human brain derived endothelial cells to pro-inflammatory cytokines IFNy and TNF $\alpha$ *in vitro*

Skander Ben Ahmed<sup>1</sup>, Olivier Tastet<sup>1</sup>, Stephanie Zandee<sup>1</sup>, Lyne Bourbonnière<sup>1</sup>, Camille Grasmuck<sup>1</sup>, Karine Thai<sup>1</sup>, Fiona Tea<sup>1</sup>, Antoine Fournier<sup>1</sup>, Romain Cayrol<sup>1</sup>, Robert Moumdjian<sup>1</sup>, Alain Bouthillier<sup>1</sup>, Martine Tétreault<sup>1,2</sup>, Alexandre Prat<sup>1,2</sup>

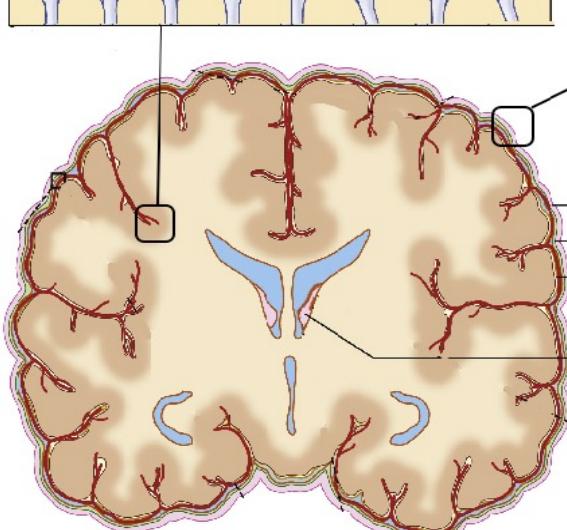
<sup>1</sup> Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CR-CHUM), QC, Canada; <sup>2</sup> Multiple sclerosis clinic, Centre Hospitalier de l'Université de Montréal (CHUM), QC, Canada

## Introduction

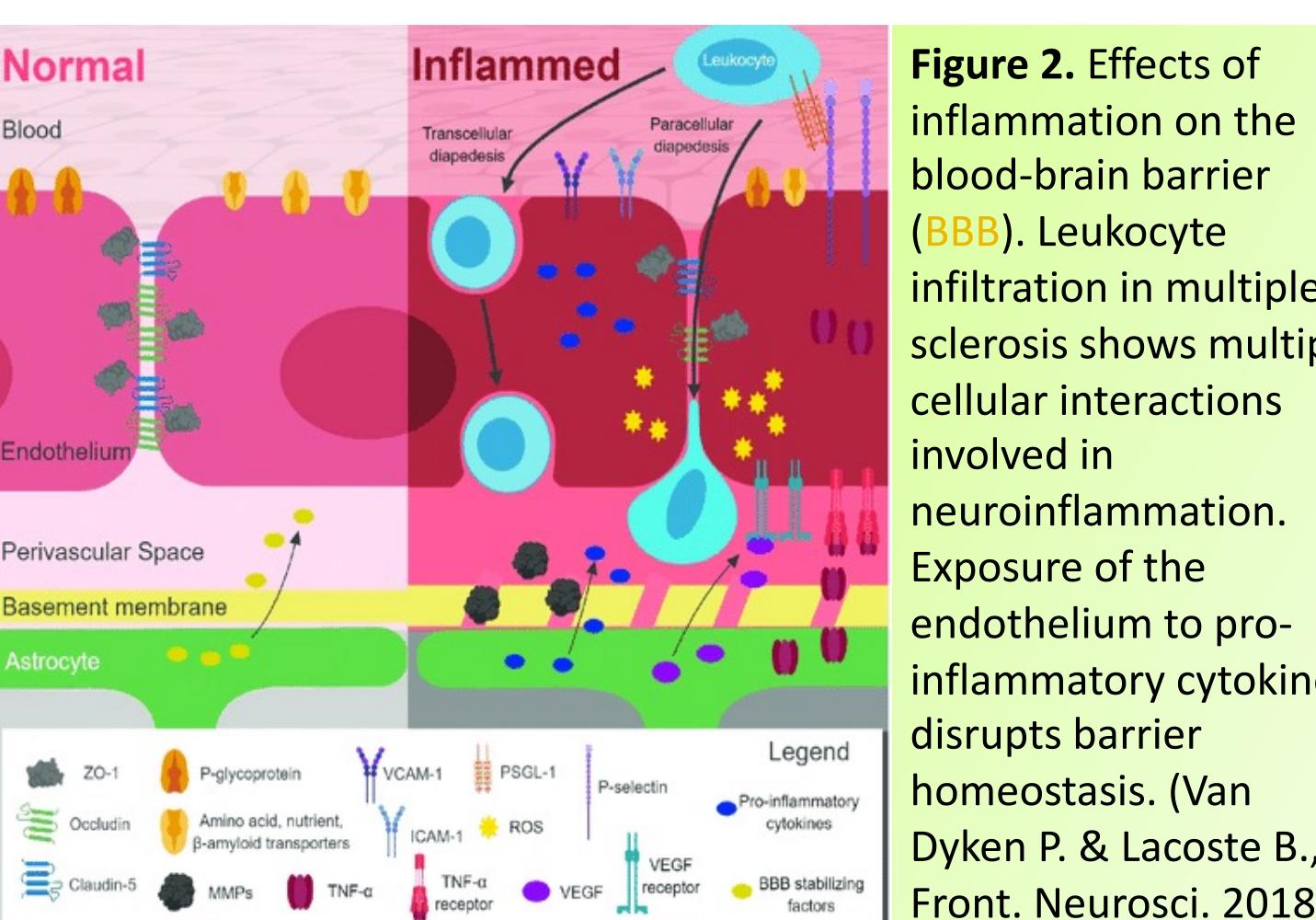
**Multiple sclerosis (MS)** is a neurodegenerative disease of the central nervous system (CNS) caused by an **autoimmune response** and chronic inflammation. Crucial physiological processes leading to the progression of the disease include the **breakdown** of the **blood-brain barrier (BBB)** and of the **meningeal brain barrier (BMB)**.



**Figure 1.** Schematic representation of immune cell trafficking through the 2 different types of brain barriers. (Charabati M., Pharmacological Sciences, 2020)



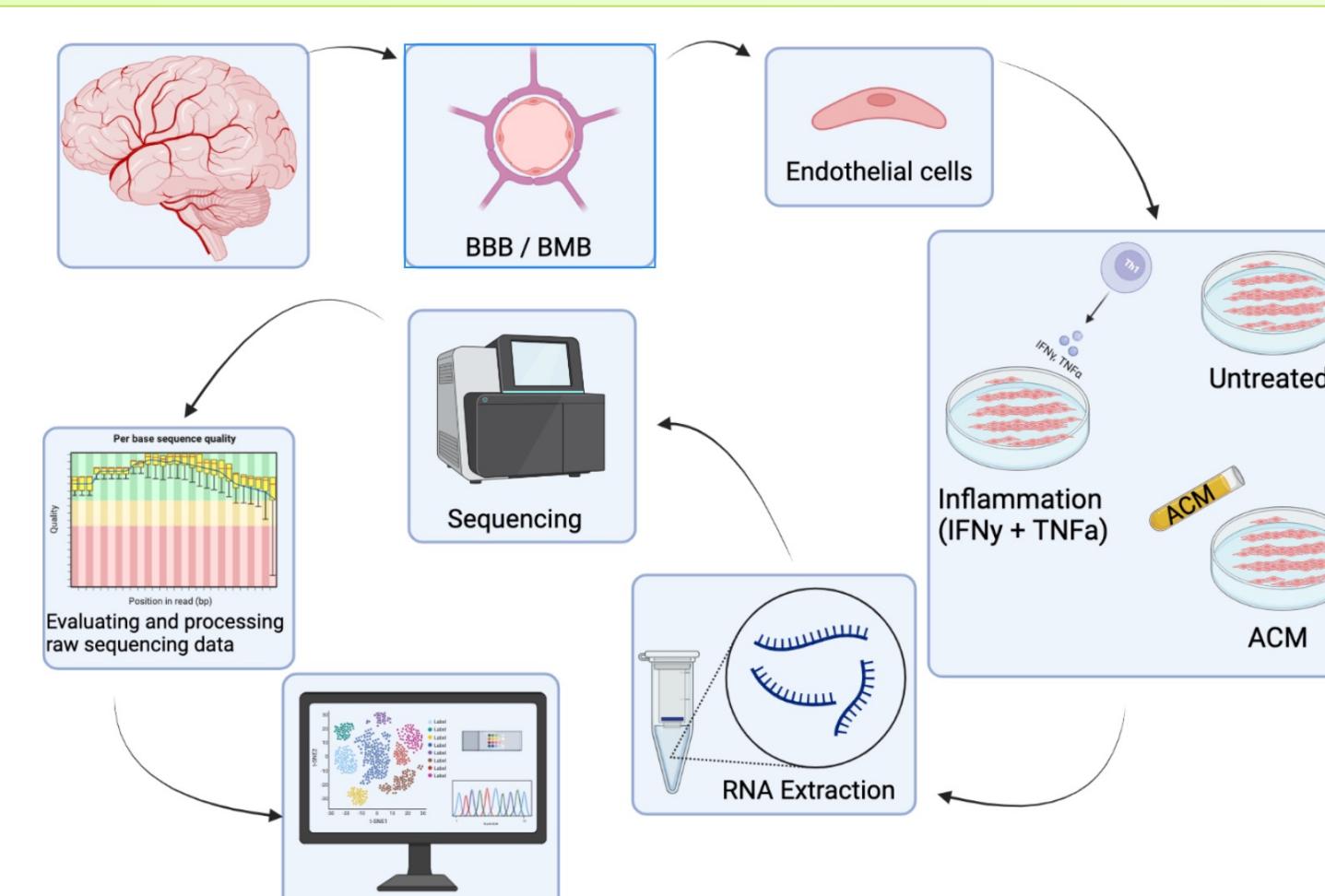
The mechanisms leading to **immune infiltration** in MS appear to involve direct effects of **cytokines/chemokines** on the **permeability of barriers** formed by endothelial cells (ECs), thereby leading to lesion formation. Exposure of the endothelium to **pro-inflammatory cytokines** interrupts the **homeostasis of barriers** by disrupting tight and adherent junctions, thereby increasing the permeability of the barrier.



**Figure 2.**

Effects of inflammation on the blood-brain barrier (BBB). Leukocyte infiltration in multiple sclerosis shows multiple cellular interactions involved in neuroinflammation. Exposure of the endothelium to pro-inflammatory cytokines disrupts barrier homeostasis. (Van Dyken P. & Lacoste B., Front. Neurosci. 2018)

**Figure 3. Workflow Outline**



Created in BioRender.com

## Hypothesis & Objectives

### Hypothesis:

- The pro-inflammatory cytokines **IFNy** and **TNF $\alpha$**  signaling secreted by different immune system effectors induces a **various functional response** in endothelial cells that are **quantifiable** through the **transcriptome**.

### Objectives:

- Compare the transcriptome of the **BBB** and the **BMB** at baseline.
- Identify **specific** and **shared responses** to inflammatory stimulation.
- Characterize the **functional response** of ECs to **IFN- $\gamma$  + TNF- $\alpha$**  stimulation using public annotations.

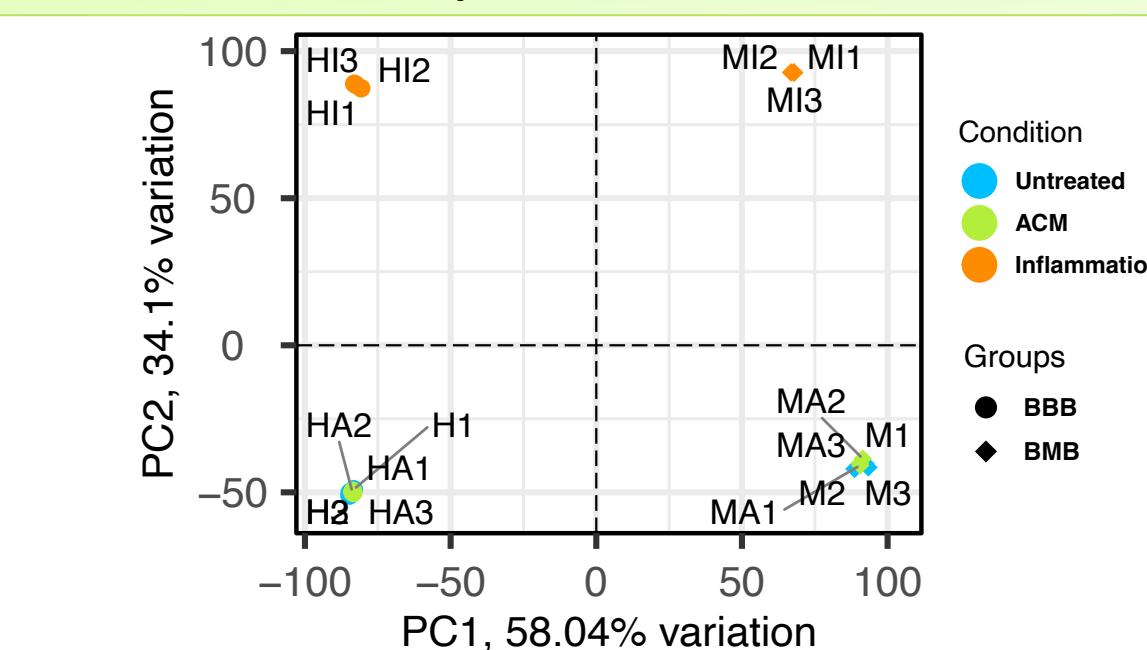
## Methods

- ECs from human brain tissue removed during surgery were **cultured** and **stimulated** with cytokines (**TNF $\alpha$**  and **IFNy**).
- Bulk RNA-Seq was performed on **untreated** and **treated** ECs on an Illumina NextSeq500 machine, at a targeted depth of 50 M reads per sample.
- Reads were **aligned** to the reference human genome using **STAR**.
- **Differential expression analysis** was performed with R package **limma**.
- **Biological information** from differentially expressed genes (DEGs) was obtained by **gene set enrichment analysis (GSEA)** and **Gene Ontology annotation (GO)**.

**Figure 3. Workflow Outline**

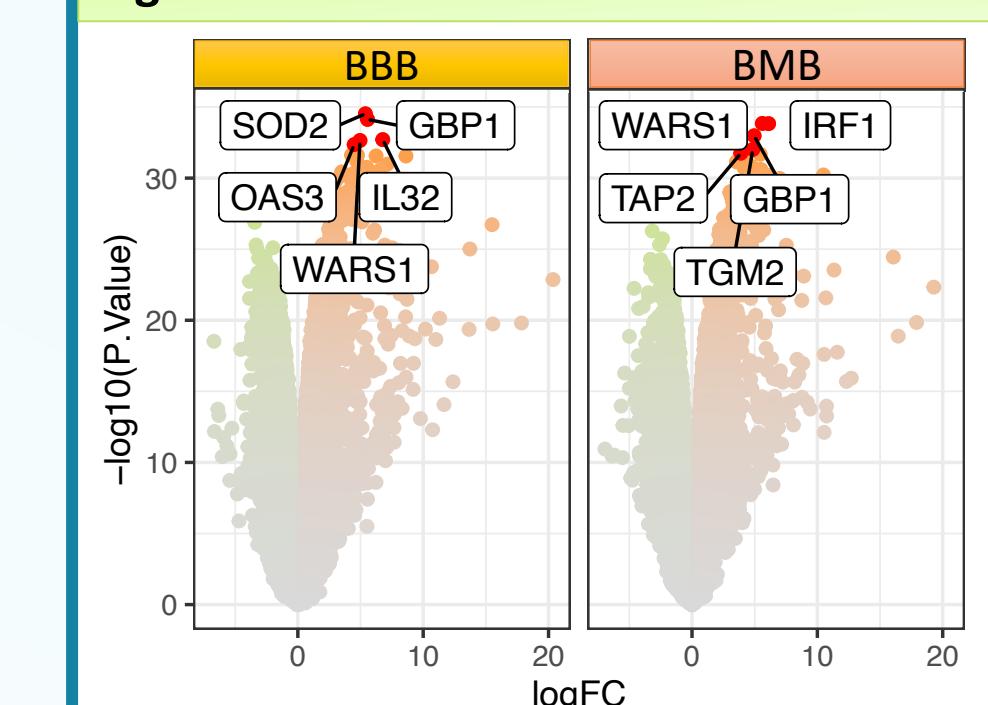
## Results

**Figure 4. PCA of the RNA-Seq data**



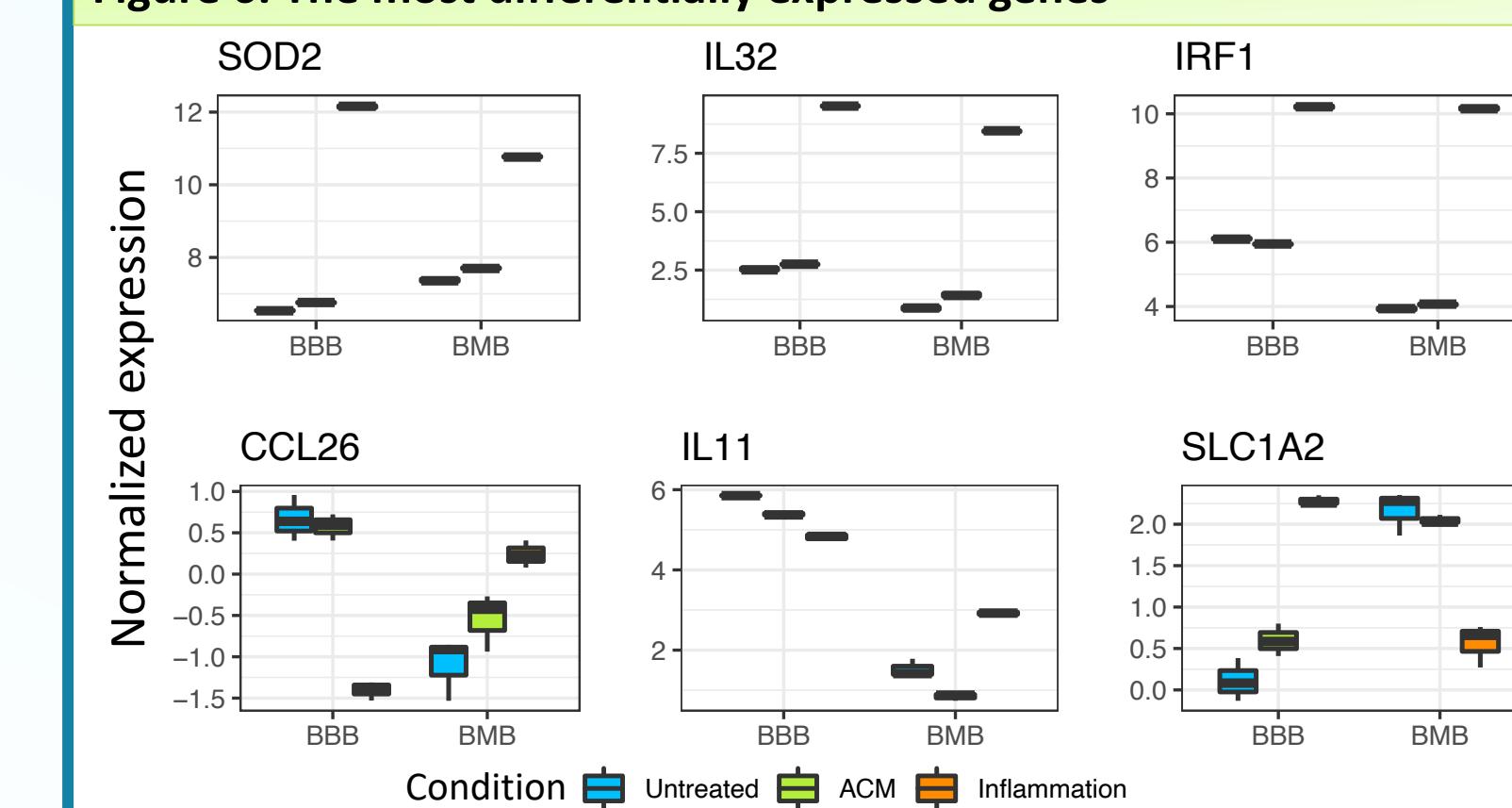
- The main axis of variation (PC1) separated the samples based on the group (**BBB/BMB**) showing **important differences between the barriers** even at baseline and the second axis of variation (PC2) is explained by the **response of ECs to the inflammatory stimulation**.
- ACM-treated and untreated showed **very little differences**, suggesting that the untreated condition simulates the normal environment of the CNS.

**Figure 5. ECs treated with ACM Vs. Inflammatory condition**



- Differentially expressed genes tend to be **upregulated** in the **inflammatory condition**.
- Cytokine stimulation induced significant transcriptional changes in **BBB/BMB**-associated ECs.

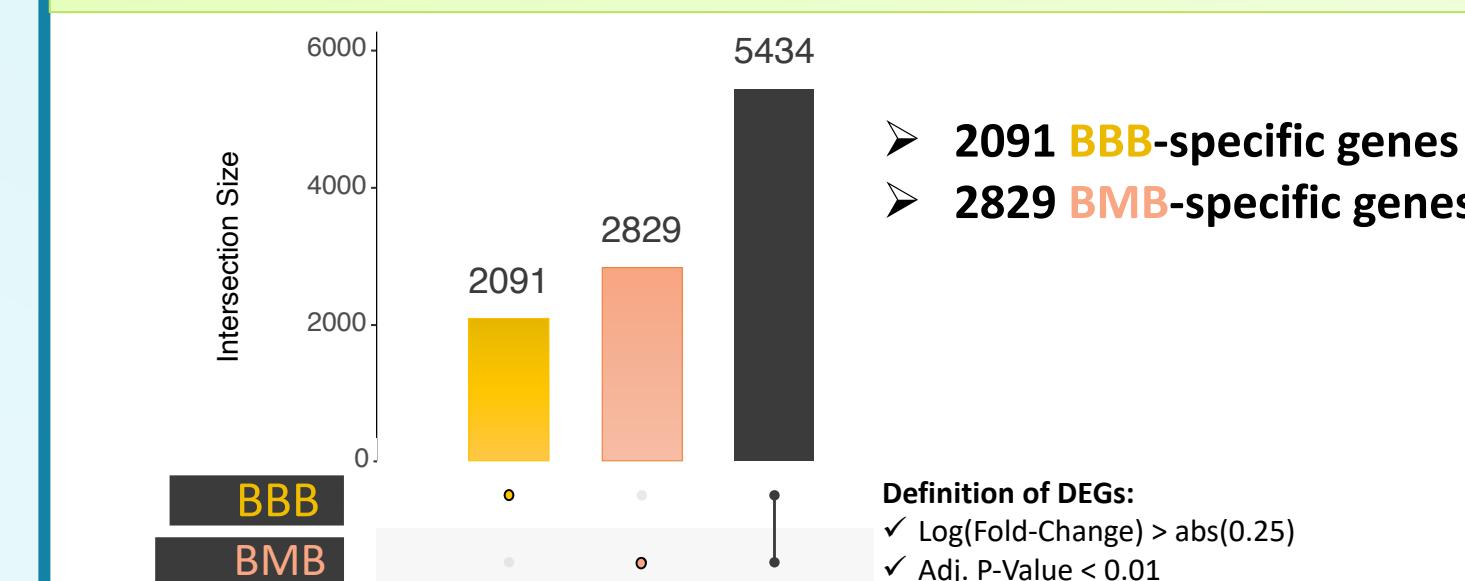
**Figure 6. The most differentially expressed genes**



- The most upregulated genes included **SOD2**, **IL32** and **IRF1** were shared across barriers.
- Barrier-specific effects were observed for **CCL26**, **IL11** and **SLC1A2**. These genes showed **opposite responses** to inflammation.

## Results

**Figure 7. Overlap of DEGs in BBB/BMB**



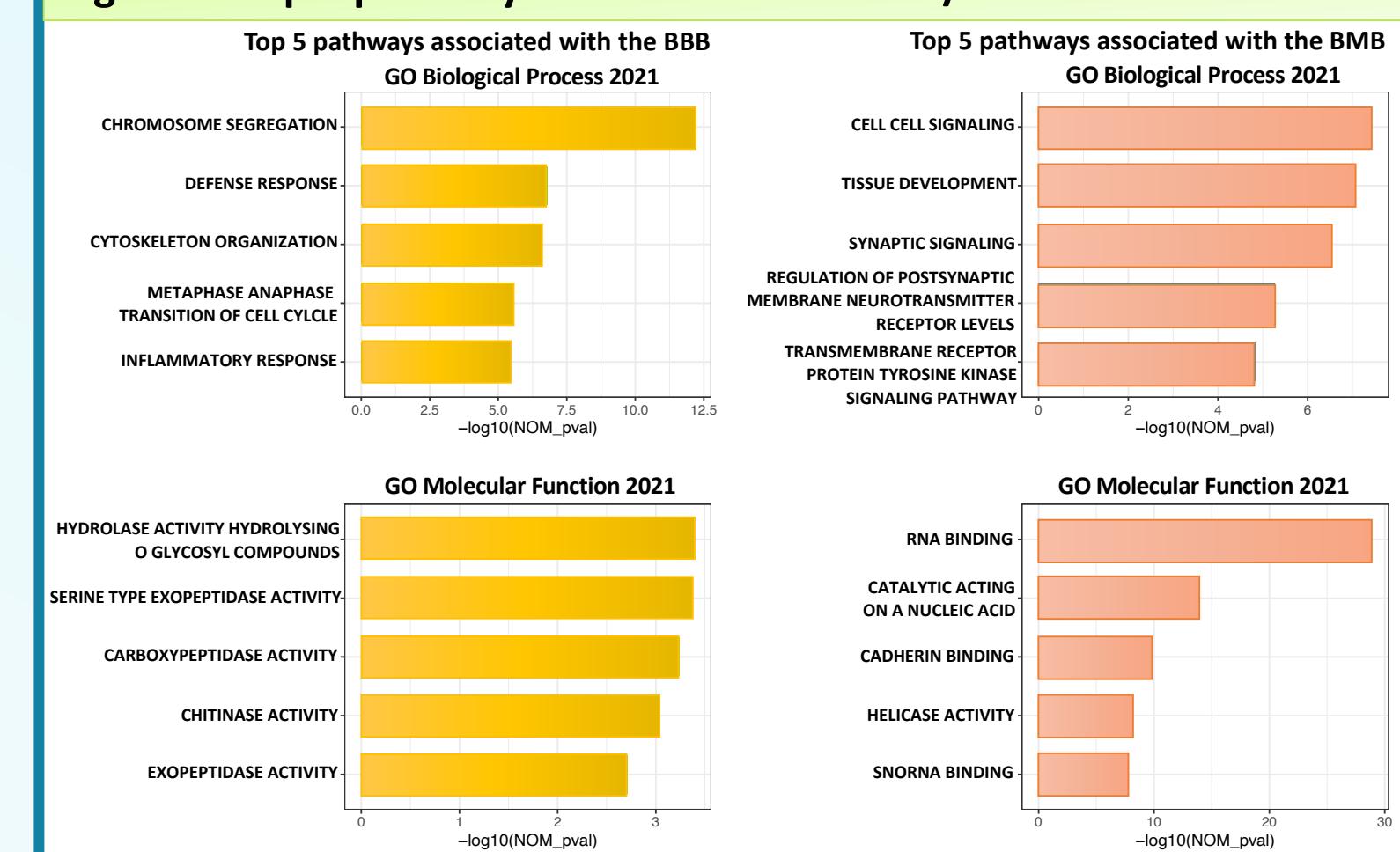
- 2091 **BBB**-specific genes
- 2829 **BMB**-specific genes

Definition of DEGs:

✓ Log(Fold-Change) > abs(0.25)

✓ Adj. P-Value < 0.01

**Figure 8. Top 5 pathways associated with BBB/BMB**



- The response of **BBB** ECs to cytokine stimulation involved pathways of **chromosome segregation** and **defense response**.
- Pathways such as **RNA binding** and **tissue development** are involved in the **BMB** response to inflammation.

## Conclusions

- Specifics transcriptional signatures to barriers demonstrated that there are **significant differences** in the **molecular properties of ECs**, suggesting differential roles for the **BBB** and the **BMB** in **immune infiltration**.
- Gene expression changes induced by cytokine stimulation showed that ECs are **responsive to cytokine stimulation**, showing they have an important role in mediating neuroinflammatory events.
- Gene ontology enrichment reveals that **inflammation** acts on various biological pathways to affect cellular physiology of ECs in the **BBB/BMB**. Transcriptional perturbations involved both immune pathways (e.g. defense response, inflammatory response) and house-keeping functions (e.g. chromosome segregation, tissue development).