# Exploring the VISTA of glial cells

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# Assessing microglia VISTA expression in CNS inflammatory and degenerative diseases using public domain RNA-sequencing data sets

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## Abstract

V-type immunoglobulin-like suppressor of T-cell activation (VISTA) is a negative checkpoint regulator (NCR) that is expressed primarily in the hematopoietic system by myeloid and T cells. NCR are intensely pursued as targets to modulate the immune response in cancer and autoimmunity. A large variety of NCR is expressed by central nervous system (CNS)-resident cell types and is associated with CNS homeostasis, interactions with peripheral immunity and CNS inflammation and disease. Immunotherapy blocking NCR affects the CNS as patients can develop neurological issues including encephalitis and multiple sclerosis (MS). VISTA regulates T-cell quiescence and activation and has a variety of functions in myeloid cells including efferocytosis, cytokine response and chemotaxis. In the CNS, VISTA is predominantly expressed by microglia and macrophages of the CNS. Here, expression of VISTA in microglia compared to other myeloid cells and during CNS diseases was analyzed using previously published mRNA sequencing datasets. VISTA was more abundantly expressed in microglia compared to peripheral myeloid cells. During neurodegenerative diseases, multiple sclerosis, stroke, and other CNS diseases and respective animal models, VISTA was generally decreased in microglia, and differentially regulated in total tissue. Understanding the role of VISTA in the CNS is important considering the adverse effects of immunotherapy on the CNS, and in view of the therapeutic potential of modulating VISTA in CNS disease.

# Main text

V-type immunoglobulin domain-containing suppressor of T-cell activation (VISTA) is a negative checkpoint regulator (NCR) that inhibits T-cell activation (Flies et al., 2011; Wang et al., 2011). VISTA is predominantly expressed by myeloid cells, but also naïve T cells (Wang et al., 2011), in which VISTA regulates T-cell quiescence (ElTanbouly et al., 2020). Since VISTA can act as a receptor and a ligand, both expression of VISTA on antigen-presenting cells (APC) and T cells can lead to inhibition of T-cell activation (Flies et al., 2011, 2014; Wang et al., 2011). VISTA has functions in myeloid cells in addition to acting as an NCR. These functions include uptake of apoptotic cells (Yoon et al., 2015; Cohen et al., 2016), cytokine response (Bharaj et al., 2014; Ceeraz, Eszterhas, et al., 2017; Ceeraz, Sergent, et al., 2017; Wang et al., 2019), and chemotaxis (Sergent et al., 2018).

*VISTA* mRNA is expressed in the brain, but relatively low in comparison to the level of *VISTA* detected in thymus, spleen, and lung (Wang et al., 2011). We previously demonstrated that in the mouse and human central nervous system (CNS), VISTA is predominantly expressed by microglia and expression levels are comparable to well-established microglia markers such as *CX3CR1*, *TMEM119*, *P2RY12*, and *ITGAM* (*CD11B*) (Borggrewe et al., 2018). Microglia are the macrophages of the CNS parenchyma and thus feature innate immune functions including phagocytosis, antigen-presentation, cytokine production, respiratory burst, and chemotaxis. In contrast to other myeloid cells, microglia also exhibit CNS-specific functions such as synaptic pruning (removal of unused synapses), release of neurotrophic factors, and support of neurogenesis (Colonna and Butovsky, 2017). Expression of most NCR is induced or upregulated in microglia and other CNS cell types during inflammation and CNS disease such as PDL1 (Yshii et al., 2017; Borggrewe et al., 2018). VISTA expression, however, is decreased in microglia during CNS inflammation and disease (Borggrewe et al., 2018). We showed that microglia VISTA expression decreases in vitro after TLR ligation using Pam3CSK4 (TLR1/2), poly I:C (TLR3), LPS (TLR4), and beta-glucan (TLR2/6, Dectin-1) (Borggrewe et al., 2018). Furthermore, microglia *VISTA* expression also decreases in microglia in vivo during experimental autoimmune encephalomyelitis (EAE), a mouse model for MS, after LPS injection as an acute inflammatory challenge, and in Ercc1 deficient mice, a DNA repair-deficient mouse model with microglia activation and accelerated ageing (Borggrewe et al., 2018). In post-mortem human multiple sclerosis (MS) tissue, VISTA expression is decreased in chronic active lesions (Borggrewe et al., 2018).

To expand on these observations, *VISTA* expression in microglia and total brain tissue was analyzed using published mRNA sequencing (RNAseq) datasets of multiple CNS diseases and respective animal models including neurodegenerative diseases (NDD), MS, infection, stroke, glioblastoma (GBM), and aging.

## VISTA expression in microglia is higher than in other myeloid cells

Among hematopoietic cells, VISTA expression is highest on myeloid cells (ElTanbouly et al., 2019). Interestingly, *VISTA* expression in microglia is higher than in other myeloid cells and other CNS-associated macrophages (Fig. 1 and Table 1). CNS myeloid cells (microglia and brain-border macrophages) express higher levels of *VISTA* than peripheral myeloid cells, and *VISTA* expression is higher in microglia compared to perivascular macrophages (Fig. 1 and Table 1). After diphtheria toxin-induced ablation of microglia expressing diphtheria toxin

receptor, *VISTA* expression is higher in repopulated microglia than in bone marrow-derived microglia (Fig. 1 and Table 1). Together these results suggest that microglia VISTA expression is higher compared to peripheral myeloid cells, which express the highest levels of VISTA among peripheral immune cells (Flies et al., 2011; Wang et al., 2011; ElTanbouly et al., 2019). Thus, microglia may express the highest levels of VISTA of any cell type in any organ.

## Microglia VISTA expression is decreased in mouse models of neurodegenerative diseases

NDD including Alzheimer's disease (AD), frontotemporal dementia (FTD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), are progressive degenerative diseases of the CNS. Hallmarks of NDD are the loss of neurons and neuroinflammation. Microglia are the major source of neuroinflammation in NDD, and significantly contribute to development and progression of these diseases (Perry and Holmes, 2014; Tang and Le, 2016; Dubbelaar et al., 2018). However, microglia also phagocytose cellular debris and plaques that are formed in many NDD, thereby facilitating clearance of waste. Hence, microglia appear to have both beneficial and detrimental functions in NDD. In the AD mouse model 5XFAD and ALS model SOD1G93A, microglia downregulate expression of homeostatic genes, while upregulating genes involved in immune activation and phagocytosis (Keren-Shaul et al., 2017). This NDD-associated microglia phenotype is also called disease-associated microglia (DAM) or microglia neurodegenerative phenotype (MGnD) (Holtman et al., 2015; Keren-Shaul et al., 2017; Krasemann et al., 2017). DAM microglia in both AD and ALS models exhibit 2-fold reduced *VISTA* expression (Fig. 2 and Table 1). The decrease in microglia *VISTA* expression is consistent across multiple AD mouse models including 5XFAD, APP/PS1, and PS2APP (Fig. 2 and Table 1). In spinal cord microglia from ALS SOD1G93A mice, *VISTA* expression is slightly upregulated in early stages, but decreased during the end stage of disease (Fig. 2 and Table 1). In tau mouse models that carry P301L or P301S mutations associated with FTD and PD, *VISTA* expression in microglia is also reduced (Fig. 2 and Table 1). Collectively,

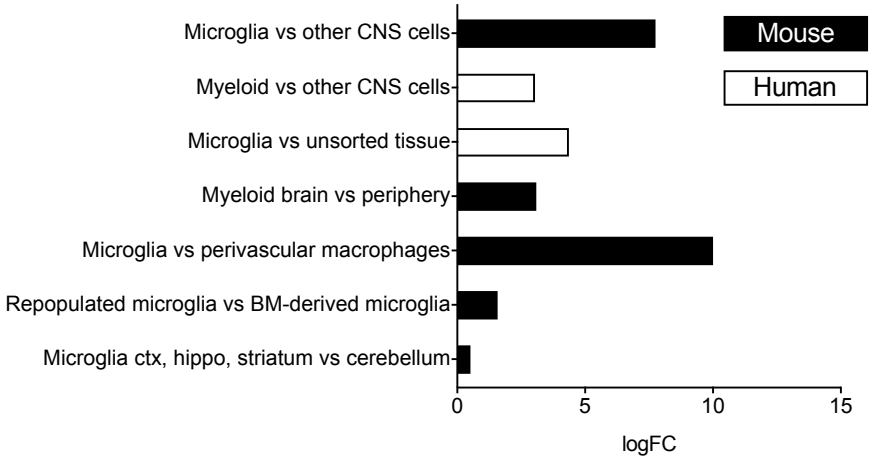


**Figure 1. VISTA expression in microglia compared to other myeloid cells.** Log Fold Change (logFC) of *VISTA* expression in microglia compared to other CNS cells, myeloid cells, bone marrow (BM)-derived microglia, and in different CNS regions (Table 1).

these data point towards *VISTA* being regulated in microglia similar to homeostatic markers, which are also decreased during microglia activation and in NDD (Dubbelaar et al., 2018). Although the function of VISTA in microglia remains unknown, VISTA knockout (KO) in myeloid cells leads to decreased phagocytosis and elevated production of cytokines (Yoon et al., 2015; Li et al., 2017; Liu et al., 2018). Therefore, reduction in microglia *VISTA* expression during NDD could have detrimental effects, as it might enhance neuroinflammation while inhibiting the clearance of cell debris and waste. Surprisingly, *VISTA* gene expression in bulk tissue from AD and ALS mice and in post-mortem human AD tissue is elevated (Fig. 2 and Table 1). Endothelial cells express low levels of *VISTA* in non-diseased conditions, but it is possible that expression is upregulated during NDD. Furthermore, *VISTA* expression might be induced in other CNS cell types in NDD, which do not express VISTA under homeostatic conditions. Together, *VISTA* expression by microglia is consistently decreased in multiple models of NDD, which could have detrimental effects. However, bulk tissue gene expression data indicates that other CNS cell types upregulate or induce *VISTA* expression in these conditions, warranting further investigation.

## VISTA is differentially expressed in MS

Microglia *VISTA* expression is reduced during all stages of actively induced EAE by myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ in complete Freund's adjuvant (CFA) (Borggrewe et al., 2018), and VISTA KO exacerbates EAE in a spontaneous TCR transgenic model (2D2) (Wang et al., 2014). Cuprizone-feeding in mice is a model in which chemical-induced death of oligodendrocytes leads to demyelination and remyelination, and microglia immune-activation in the absence of peripheral immune cell infiltrates. *VISTA* expression in microglia is also reduced in this MS mouse model (Fig. 2 and Table 1). Furthermore, VISTA expression is decreased in chronic active MS lesions (Borggrewe et al., 2018), jointly suggesting a role for VISTA in MS.



Most MS lesions occur in WM; however, GM lesions are frequent and are a hallmark of MS. In MS WM, microglia *VISTA* expression is slightly decreased compared to WM of non-demented controls (NDC), whereas no difference is evident in MS GM (Fig. 2 and Table 1). A hallmark of MS lesions and EAE is the infiltration of peripheral immune cells including macrophages and lymphocytes. More recently, neutrophils were also associated with lesion formation and MS pathology (Pierson et al., 2018). A loss or reduction of VISTA expression on microglia in MS and EAE may boost (re)activation of infiltrating T cells in lesions, thereby exacerbating inflammation and tissue damage. Moreover, reduced VISTA levels in microglia and infiltrating monocytes may impair their phagocytic ability, which is important for clearance of cellular and myelin debris early during the disease (Voet et al., 2019). The role of VISTA in microglia in MS and EAE might depend on the stage of disease and the type of MS lesion, including the lesion microenvironment and how microglia respond to these environmental cues. Microglia-specific gene expression in different types of MS lesions has not been studied yet, however, data on bulk tissue from different lesions is available. Here, *VISTA* expression is upregulated in all investigated types of lesions including inactive, active, chronic active, and remyelinated (Fig. 2 and Table 1). It remains conceivable that microglia *VISTA* expression is reduced, but this cannot be detected in bulk tissue when other cell types upregulate or induce *VISTA* expression. As discussed above, although endothelial VISTA expression is low under homeostatic conditions, it might be upregulated during non-homeostatic conditions. Furthermore, other CNS cell types may induce VISTA expression,

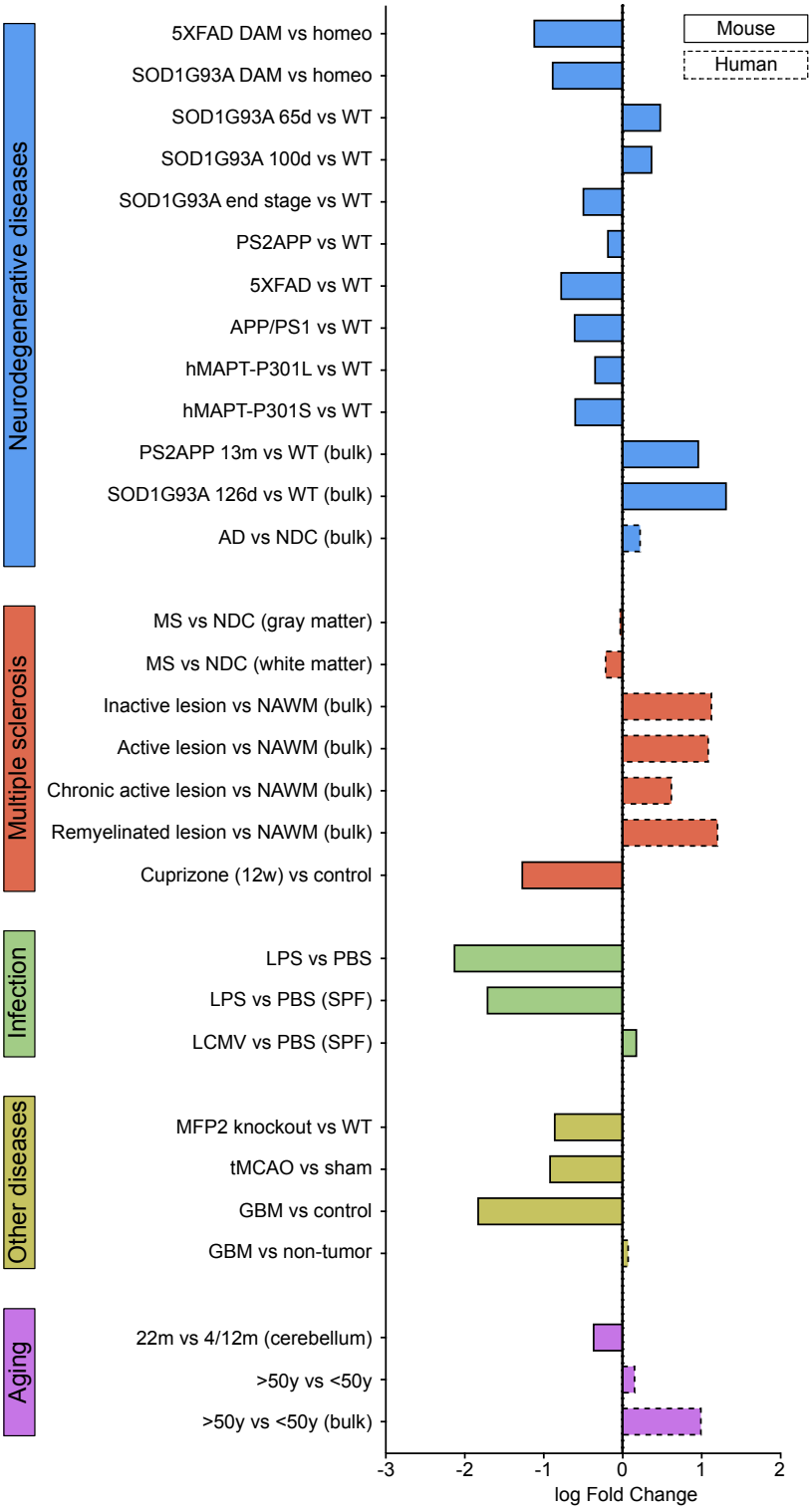


**Figure 2. VISTA expression in CNS diseases and aging.** Log Fold Change of *VISTA* expression in microglia or bulk CNS tissue during disease compared to control (Table 1). Homeo = homeostatic microglia, WT = wildtype, NDC = non-demented control, NAWM = normal-appearing white matter, PBS = phosphate buffered saline, LCMV = lymphocytic choriomeningitis virus, SPF = specific-pathogen free, MFP2 = multifunctional protein-2, tMCAO = transient middle cerebral artery occlusion

explaining elevated *VISTA* levels in bulk tissue. *VISTA* is likely also expressed by infiltrating immune cell subsets including neutrophils, lymphocytes, and myeloid cells in MS lesions. VISTA is upregulated at least in myeloid cells under inflammatory conditions (Bharaj et al., 2014), which would also explain elevated *VISTA* levels in MS lesions. It will be important to assess cell type-specific VISTA expression in different types of MS lesions to dissect the role of VISTA in microglia and other cell types during MS development and progression.

## Microglia VISTA decreases after LPS stimulation in mice

Microglia express a range of pattern-recognition receptors such as TLR, C-type lectin receptors, and NOD-like receptors, which allows them to sense and respond to pathogen-associated and damage-associated molecular patterns (Rock et al., 2004). In the CNS, microglia are the major cell type capable of monitoring and defending the tissue from intruders including bacteria and viruses. Upon response towards microbial compounds such as LPS (TLR4), poly I:C (TLR3), beta-glucan (Dectin-1, TLR2/6), Pam3CSK4 (TLR1/2), *VISTA* expression decreases in mouse and rhesus macaque microglia in vitro by 50-70% (Borggrewe et al., 2018). A similar decrease is observed in mouse microglia 3/6/24 hours after intraperitoneal LPS injection (Borggrewe et al., 2018) (Fig. 2 and Table 1). Although VISTA expression is reduced after LPS injection, it is not altered during infection with lymphocytic choriomeningitis virus (LCMV) by intracerebral inoculation (Fig. 2 and Table 1). The lack of studies on infections in relation to VISTA biology in the CNS underscores that this important topic remains largely unexplored.

## Microglia VISTA expression is mostly decreased in other CNS diseases

By contributing to neuroinflammatory mechanisms, microglia are also involved in a range of other neurological diseases including stroke, cancer, and more. *VISTA* expression by microglia is reduced in almost all CNS disease conditions including multifunctional protein-2 (MFP2) KO mice (Fig. 2 and Table 1). MFP2 defects in humans usually lead to severe developmental pathologies including neonatal hypotonia, seizures, psychomotor retardation, and brain malformations (Verheijden et al., 2014). In mice, MFP2 KO leads to Purkinje cell degeneration and neuroinflammation (Verheijden et al., 2014). During transient middle-cerebral artery occlusion (tMCAO), which leads to stroke in mice, microglia *VISTA* expression is reduced 2-fold (Fig. 2 and Table 1). Although inhibition of microglia activation during stroke leads to beneficial outcomes, microglia activation is also necessary to counteract neuronal death and enhance neurogenesis (Qin et al., 2019). Microglia and macrophages are part of the tumor environment in GBM and promote tumor progression by producing anti-inflammatory cytokines, immunosuppressive molecules, and angiogenic factors (Matias et al., 2018). Although microglia acquire a more immune-silencing phenotype characterized by secretion of anti-inflammatory cytokines and an upregulation of NCR, *VISTA* expression is reduced in mouse microglia and unaltered in human microglia associated with GBM (Fig. 2 and Table 1). A decrease in *VISTA* expression may be beneficial for GBM, since KO of VISTA renders mice highly resistance against glioma tumors (Flies et al., 2014). During aging, microglia are thought to become primed, dystrophic, and senescent, leaving them less responsive and uncapable of properly monitoring the CNS (Spittau, 2017); hence, microglia phenotypes associated with aging may contribute to the development of NDD such as AD and PD. Aged mouse cerebellar microglia exhibit reduced *VISTA* expression compared to microglia from younger mice (Fig. 2 and Table 1). In humans, such a comparison is more difficult due to limited availability of

post-mortem tissue from young individuals. However, *VISTA* expression is slightly increased in microglia from individuals >50 years of age compared to <50 years (Fig. 2 and Table 1). This increase is much more pronounced in bulk tissue, which again supports the notion that other cell types may upregulate or induce VISTA upon deficits in CNS homeostasis.

## Concluding remarks

VISTA is an NCR with unique characteristics, in the CNS predominantly expressed by microglia. Expression of VISTA is decreased in microglia during ageing, neuroinflammation, and multiple CNS diseases including neurodegeneration, MS, stroke, and cancer. VISTA is involved in inhibition of T-cell activation, hence a lack of VISTA in the presence of infiltrating immune cells, such as in MS, may promote inflammation. Microglia VISTA expression is very high and since T cells are mostly absent in the CNS parenchyma during health and in most diseases, it is conceivable that VISTA has functions in microglia in addition to inhibiting T-cell activation. In other myeloid cell types, VISTA is involved in uptake of apoptotic cells (Yoon et al., 2015; Cohen et al., 2016), cytokine response (Bharaj et al., 2014; Ceeraz, Eszterhas, et al., 2017; Ceeraz, Sergent, et al., 2017; Wang et al., 2019), and chemotaxis (Sergent et al., 2018). Generally, VISTA KO promotes a pro-inflammatory phenotype in myeloid cells and in mouse models of inflammation. Thus, a reduced VISTA expression in microglia may impart a more pro-inflammatory phenotype and as a consequence amplify neuroinflammation during CNS disease. It is essential to assess the exact function of VISTA in microglia to allow definitive conclusions how a decrease in microglia VISTA expression may affect CNS disease etiology. Furthermore, the effect of a loss of VISTA in the CNS on neuroinflammation should be evaluated, since modulating VISTA signaling may offer new strategies for therapeutic targeting. If reduced VISTA expression promotes inflammation, restoring VISTA function in microglia could be beneficial for CNS diseases. More knowledge on the functions of VISTA in the CNS and the effects of VISTA modulation on the CNS will help to evaluate the therapeutic potential of targeting VISTA in CNS diseases.

**Table 1. *VISTA* expression in microglia, CNS diseases, and ageing**

| | Description | Species | Tissue | Cell subset | Condition | logFC | padj | Reference | |
|---|---|---|---|---|---|---|---|---|---|
| *Microglia in healthy CNS* | Microglia vs other CNS cells | Mouse | Cortex | $CD45^{pos}$ | Control | 7.75 | 0.006 | (Zhang et al., 2014) | * |
| | Myeloid vs other CNS cells | Human | Temporal cortex | $CD45^{pos}$ | Control | 3.05 | 0.001 | (Zhang et al., 2016) | * |
| | Microglia vs unsorted tissue | Human | Cortex | $CD11B^{pos}$ $CD45^{int}$ | Control | 4.06 | 0.000 | (Galatro et al., 2017) | |
| | Myeloid brain vs periphery | Mouse | Brain, peripheral tissues | $CD11B^{pos}$ $CD45^{int}$ | Control | 3.09 | 0.015 | (Lavin et al., 2014) | * |
| | Microglia vs perivascular macrophages | Mouse | Somatosensory cortex, CA1 hippocampus | Myeloid (scRNAseq) | Control | 10.00 | 1.000 | (Zeisel et al., 2015) | * |
| | Repopulated microglia vs BM-derived microglia | Mouse | Brain | $CD11B^{pos}$ $CD45^{int}$ | Control | 1.58 | 0.001 | (Bruttger et al., 2015) | * |
| | Microglia ctx, hippo, striatum vs cerebellum | Mouse | Cerebellum, cortex, hippocampus, striatum | $CD11B^{pos}$ | Control | 0.51 | 0.000 | (Grabert et al., 2016) | * |
| *Neurodegenerative diseases* | 5XFAD DAM vs homeo | Mouse | Brain | $CD45^{pos}$ | 5XFAD | -1.13 | NA | (Keren-Shaul et al., 2017) | |
| | SOD1G93A DAM vs homeo | Mouse | Spinal cord | $CD45^{pos}$ | SOD1G93A | -0.90 | NA | (Keren-Shaul et al., 2017) | |
| | SOD1G93A 65d vs WT | Mouse | Spinal cord | $CD11B^{pos}$ | SOD1G93A | 0.49 | NA | (Chiu et al., 2013) | |
| | SOD1G93A 100d vs WT | Mouse | Spinal cord | $CD11B^{pos}$ | SOD1G93A | 0.38 | NA | (Chiu et al., 2013) | |
| | SOD1G93A end stage vs WT | Mouse | Spinal cord | $CD11B^{pos}$ | SOD1G93A | -0.51 | NA | (Chiu et al., 2013) | |
| | PS2APP vs WT | Mouse | Cortex | Cx3cr1::Gfp+ | PS2APP | -0.20 | 0.496 | (Friedman et al., 2018) | * |
| | 5XFAD vs WT | Mouse | Brain | $CD11B^{pos}$ $CD45^{int}$ | 5XFAD | -0.79 | 0.078 | (Wang et al., 2015) | * |
| | APP/PS1 vs WT | Mouse | Cortex | $CD11B^{pos}$ $CD45^{int}$ | APP/PS1 | -0.62 | 0.000 | (Orre et al., 2014) | * |
| | hMAPT-P301L vs WT | Mouse | Hippocampus | $CD11B^{pos}$ | hMAPT-p301L | -0.36 | 0.608 | (Friedman et al., 2018) | * |
| | hMAPT-P301S vs WT | Mouse | Hippocampus | $CD11B^{pos}$ | hMAPT-p301S | -0.61 | 0.124 | (Friedman et al., 2018) | * |
| | PS2APP 13m vs WT (bulk) | Mouse | Cortex | Bulk tissue | PS2APP | 0.97 | 0.000 | (Srinivasan et al., 2016) | * |
| | SOD1G93A 126d vs WT (bulk) | Mouse | Spinal cord | Bulk tissue | SOD1G93A | 1.32 | 0.007 | (Lerman et al., 2012) | * |
| | AD vs NDC (bulk) | Human | Fusiform gyrus | Bulk tissue | AD | 0.23 | 0.150 | (Friedman et al., 2018) | * |
| *Multiple sclerosis* | MS vs NDC (gray matter) | Human | Gray matter | $CD15^{neg}$ $CD11B^{pos}$ | MS | -0.04 | NA | (van der Poel et al., 2019) | |
| | MS vs NDC (white matter) | Human | White matter | $CD15^{neg}$ $CD11B^{pos}$ | MS | -0.23 | NA | (van der Poel et al., 2019) | |
| | Inactive lesion vs NAWM (bulk) | Human | White matter | Bulk tissue | MS | 1.14 | NA | (Elkjaer et al., 2019) | |
| | Active lesion vs NAWM (bulk) | Human | White matter | Bulk tissue | MS | 1.10 | NA | (Elkjaer et al., 2019) | |
| | Chronic active lesion vs NAWM (bulk) | Human | White matter | Bulk tissue | MS | 0.63 | NA | (Elkjaer et al., 2019) | |
| | Remyelinated lesion vs NAWM (bulk) | Human | White matter | Bulk tissue | MS | 1.22 | NA | (Elkjaer et al., 2019) | |
| | Cuprizone (12w) vs control | Mouse | Brain | $CD11B^{pos}$ $CD45^{int}$ | Cuprizone | -1.28 | 0.424 | (Poliani et al., 2015) | * |
| *Infection* | LPS vs PBS | Mouse | Cortex | $CD11B^{pos}$ | LPS | -2.14 | 0.000 | (Srinivasan et al., 2016) | * |
| | LPS vs PBS (SPF) | Mouse | Brain | $CD11B^{pos}$ $CD45^{int}$ | SPF, LPS | -1.72 | 0.363 | (Erny et al., 2015) | * |
| | LCMV vs PBS (SPF) | Mouse | Brain | $CD11B^{pos}$ $CD45^{int}$ | SPF, LCMV | 0.19 | 0.918 | (Erny et al., 2015) | * |
| *Other diseases* | MFP2 KO vs WT | Mouse | Brain | $CD11B^{pos}$ $CD45^{int}$ | MFP2 KO | -0.87 | 0.091 | (Verheijden et al., 2015) | * |
| | tMCAO vs sham | Mouse | Cortex | $CD11B^{pos}$ $CD45^{int}$ | tMCAO | -0.93 | 0.414 | (Arumugam et al., 2017) | * |
| | GBM vs control | Mouse | Brain, tumour | $CD11B^{pos}$ | Glioma | -1.84 | 0.002 | (Szulzewsky et al., 2015) | * |
| | GBM vs non-tumour | Human | Brain, tumour | Microglia (scRNAseq) | Glioma | 0.08 | NA | (Darmanis et al., 2017) | |
| *Ageing* | 22m vs 4/12m (cerebellum) | Mouse | Cerebellum | $CD11B^{pos}$ | Control | -0.38 | 0.161 | (Grabert et al., 2016) | * |
| | >50y vs <50y | Human | Cortex | $CD11B^{pos}$ $CD45^{int}$ | Control | 0.16 | NA | (Galatro et al., 2017) | |
| | >50y vs <50y (bulk) | Human | Cortex | $CD11B^{pos}$ $CD45^{int}$ | Control | 1.00 | NA | (Galatro et al., 2017) | |

* Data from myeloid brain expression meta-analysis (Friedman et al., 2018), BM = bone marrow, ctx = cortex, hippo = hippocampus, DAM = disease-associated microglia, Homeo = homeostatic microglia, WT = wildtype, AD = Alzheimer's disease, MS = Multiple sclerosis, tMCAO = transient middle cerebral artery occlusion, SPF = specific pathogen free, LPS = lipopolysaccharide, LCMV = lymphocytic choriomeningitis virus, MFP2 = multifunctional protein-2, GBM = glioblastoma, KO = KO, NA = not available