Supplementary Figure 1
Supplementary Figure 2
Supplementary Figure 3
Supplementary Figure 4.
Supplementary Figure 5
Supplementary Figure 6

Expression (arcsinh)

C1
C4
C5
C7
C8
C9
C10
C11

CD45
TNF
IL-6
CD44
CXCR3
IL-10
CD66b
CCL2
CD3
IRF4
CD130
cyclinB1
CD56
CD61
IFN-α
CD11c
CD8a
CD14
CD86
EMR1
HLA-DR
CD19
P2Y12
TREM2
MS4A4A
Clec12a
CD68
CD33
Clec12a

NAWM
Lesion
Supplementary Figure 7
Supplementary Figure 8.
Supplementary Figure 9.
Supplementary Figure 11
**Figure legends**

**Supplementary Figure 1** Flow cytometry analysis of microglia isolated from MS brain donors. Representative dot plots showing about 50% of total events were cells (SSC-A vs FSC-A) of which more than 90% are cell-singlets (FSC-W vs FSC-H and SSC-W vs SSC-H). Of note, these cell-singlets were identified as DNA (\(^{191/193}\text{Ir}\))-positive in CyTOF, whereas the other events were DNA-negative. More than 70% of detected single cells were viable (Live/Dead vs FCS-A) and > 95% are myeloid cells (CD45+CD3-CD19- and CD45+CD11b+).

**Supplementary Figure 2** MS lesion characterization. (a) Representative brain sections of control white matter (CON, upper image; scale bar = 1 mm) and normal appearing white matter (NAWM) as well as lesion-enriched white matter (Lesion) of donors with MS pathology (lower image; scale bar = 1 mm). NAWM tissue shows ramified microglia and intact myelin, based on HLA-DR and PLP staining, respectively. In active lesion, demyelinated center and active microglia/macrophages could be detected throughout the whole lesion. The majority of HLA-DR+ cells in lesion MS are foamy microglia/macrophages. Scale bar of high resolution pictures is 50 \(\mu\)m. (b) Isolated IRF8+ nuclei, enriched for microglia genes show conserved expression of homeostatic genes CX3CR1, TMEM119, P2RY12 and ADGRG1 in active MS lesions (n=5) as compared to NAWM (n=7), detected by RT-qPCR. (c) Microglial expression of CX3CR1, P2Y12 and GPR56 is also not changed at protein level in active MS lesions (n=4-8) compared to NAWM (n=4-8), detected by flow cytometry. Statistical testing: Mann Whitney test and Wilcoxon paired t-test. (d) Representative laser confocal microscopic images of CON, NAWM and lesion WM tissues showing microglia as P2Y\(_{12}^+\) cells (green), and nuclei are counterstained with DAPI (blue). The graph shows the quantification of P2Y\(_{12}^+\) cells in CON, NAWM and lesion WM tissues. Statistical testing: Brown-Forsythe and Welch ANOVA test; * \(p < 0.05\) and ** \(p < 0.01\).
Supplementary Figure 3  Immunohistochemistry analysis of P2Y$_{12}^{+}$ cells.
Representative laser confocal microscopic images of NAWM and lesion WM tissues showing
CD68 (red, a) and HLA-DR (red, b) expression on P2Y$_{12}^{+}$ (green) microglia, and nuclei are
counterstained with DAPI (blue). The graph shows the quantification of CD68$^+$P2Y$_{12}^{+}$ (a) and
HLA-DR$^+$P2Y$_{12}^{+}$ (b) cells in NAWM and lesion WM tissues. Statistical testing: Mann-Whitney
test; $p < 0.05$ was considered as statistically significant.

Supplementary Figure 4  Marker expression-Exp-I. The overlaid t-SNE plot of 8 NAWM
and 7 lesion white matter samples. The 2D t-SNE maps were generated based on expression
levels of all markers of Exp-I (Supplementary Table 8), each dot represents a cell and the color
denotes the expression level of each targeted protein.

Supplementary Figure 5  TREM2 expression. Dot plot graph show TREM2 expression
(Exp-I) of hoMG C3 cluster compared to the lower abundant C8 and enriched clusters C1 and
C11 in active lesions.

Supplementary Figure 6  Cluster-wise assessment of phenotypic differences (Exp-I).
The graphs showed differential marker expression (mean ± sd) between myeloid cells from
NAWM and lesion WM tissues within each defined cluster. Small phenotypic differences
between the studied groups were found in defined cluster C1, C4, C5, C7, C8, C9, C10 and
C11, whereas no significant differences in marker expression were observed in C2, C3, C6,
and C12. Statistical testing: Mann-Whitney test; * $p < 0.05$ and ** $p < 0.05$.

Supplementary Figure 7  Analysis of myeloid cell heterogeneity using imaging CyTOF.
(a) Representative IMC images of normal-appearing white matter (NAWM) and Lesion WM
(scale bar = 200 µm (low resolution) and 50 µm (high resolution)) show expression of 13 markers analyzed. (b) Heat map demonstrates the phenotypic diversity of all clusters characterized using PhenoGraph. The heat color shows expression levels of all markers used for the analysis.

**Supplementary Figure 8**  **Marker expression-Exp-II.** The overlaid t-SNE plot of 10 NAWM and 8 lesion white matter samples. The 2D t-SNE maps were generated based on expression levels of all markers of Exp-II (Supplementary Table 8), each dot represents a cell and the color denotes the expression level of each targeted protein.

**Supplementary Figure 9**  **Marker expression-Exp-III.** The overlaid t-SNE plot of 8 NAWM and 9 lesion white matter samples. The 2D t-SNE maps were generated based on expression levels of all markers of Exp-III (Supplementary Table 8), each dot represents a cell and the color denotes the expression level of each targeted protein.

**Supplementary Figure 10**  **FlowSOM phenotypic heat maps – Exp-II and -III.** Heat map cluster demonstrates the expression levels of all markers used for the cluster analysis in Exp-II (a) and Exp-III (b). Heat colors of expression levels have been scaled for each marker individually (to the 1st and 5th quintiles) (red, high expression; blue, low expression).

**Supplementary Figure 11**  **Cluster-wise assessment of phenotypic differences (Exp-II).** The graphs showed differential marker expression (mean ± sd) between myeloid cells from NAWM and lesion WM tissues within each defined cluster. Small phenotypic differences between the studied groups were found in cluster C1, C3, C4, C6, C8 and C11, whereas strongly decreased expressions of CD116, NFAT1, CD44 and GM-CSF in lesion-associated myeloid cells were detected in cluster C12. No significant differences in marker expression
were observed in C2, C5, C7, C9 and C10. Statistical testing: Mann-Whitney test; * p < 0.05 and ** p < 0.05.

Supplementary Figure 12  Cluster-wise assessment of phenotypic differences (Exp-III).
The graphs showed differential marker expression (mean ± sd) between myeloid cells from NAWM and lesion WM tissues within each defined cluster. Small phenotypic differences between the studied groups were found in cluster C1, C2, C3, C4, C5, C6, C7, C9 and C11, whereas no significant differences in marker expression were observed in C8, C10 and C12. Statistical testing: Mann-Whitney test; * p < 0.05 and ** p < 0.05.