

Supplementary Data of the Results

“Profiling microglia in a mouse model of Machado-Joseph disease”

by Campos et al., 2022

Contents within the present file:

1. Supplementary Figures:

Suppl. Figure S1. Confirmation of the high purity of microglia in culture.

Suppl. Figure S2. Expression of mutant *ATXN3* in microglia from CMVMJD135 mice at two different time points in culture.

Suppl. Figure S3. Microglia expressing *ATXN3* showed a less activated phenotype in response to lipopolysaccharides (LPS) in artificially “aged” primary cultures.

Suppl. Figure S4. CMVMJD135 and Wild-Type (WT)-derived microglia showed an increased phagocytic efficiency in the presence of LPS in culture.

Suppl. Figure S5. The ramification state of microglia in the pontine nuclei (PN) of the CMVMJD135 mice is similar to those of microglia from WT mice.

Suppl. Figure S6. The complexity and shape of microglia in the PN of CMVMJD135 mice are similar to those of microglia from WT mice.

Suppl. Figure S7. Microglia in the deep cerebellar nuclei (DCN) of CMVMJD135 mice showed no differences in features relevant to microglia ramification.

Suppl. Figure S8. Microglia in the DCN of CMVMJD135 mice showed no changes in the complexity and shape.

Suppl. Figure S9. Some parameters associated with microglia ramification were similar between CMVMJD135 and WT mice in the cervical spinal cord (CSC).

Suppl. Figure S10. No changes were observed in the parameters related to the complexity of ramifications and with the cylindrical shape of the cells between groups in the CSC.

Suppl. Figure S11. Evaluation of microglial enrichment in RNA-sequencing samples.

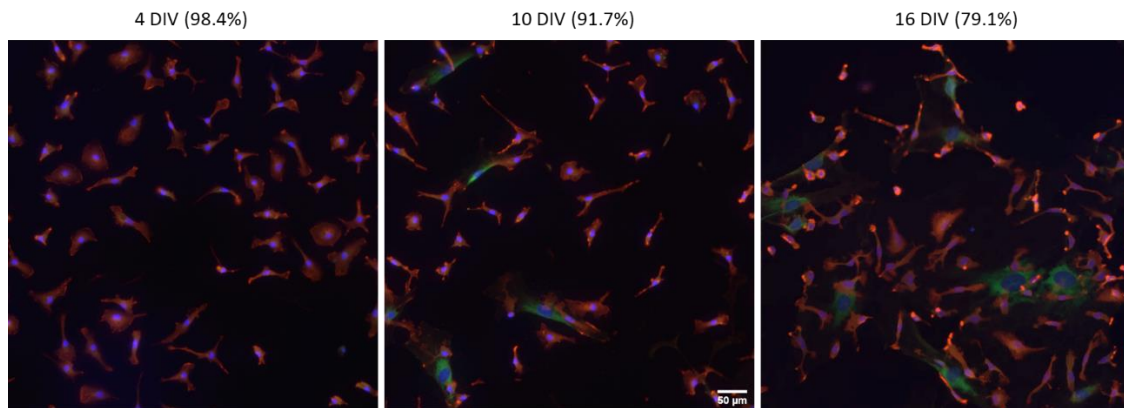
Suppl. Figure S12. Differential gene expression between microglia from CMVMJD135 and WT mice.

Suppl. Figure S13. Transcriptional changes seen in CMVMJD135 microglia overlap those in Amyotrophic lateral sclerosis (ALS) and Alzheimer disease (AD) mouse models.

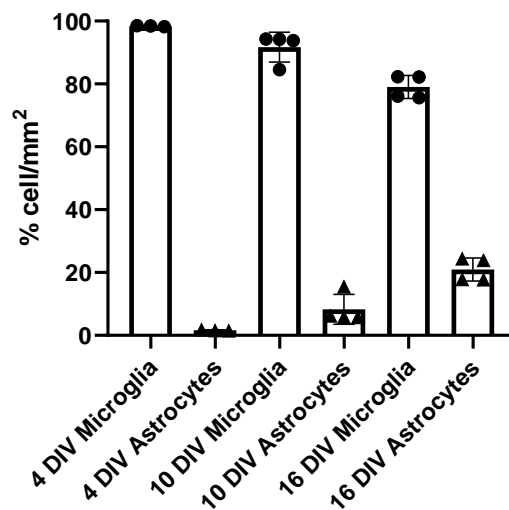
2. Supplementary References

Purity assessment of microglia culture over time

a)

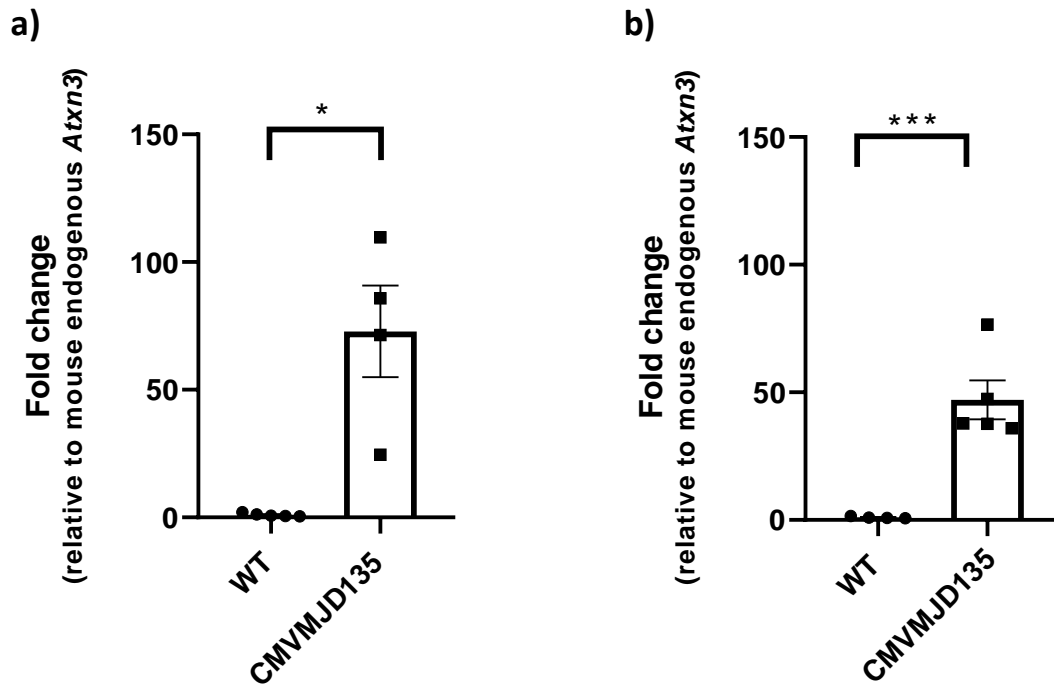


b)



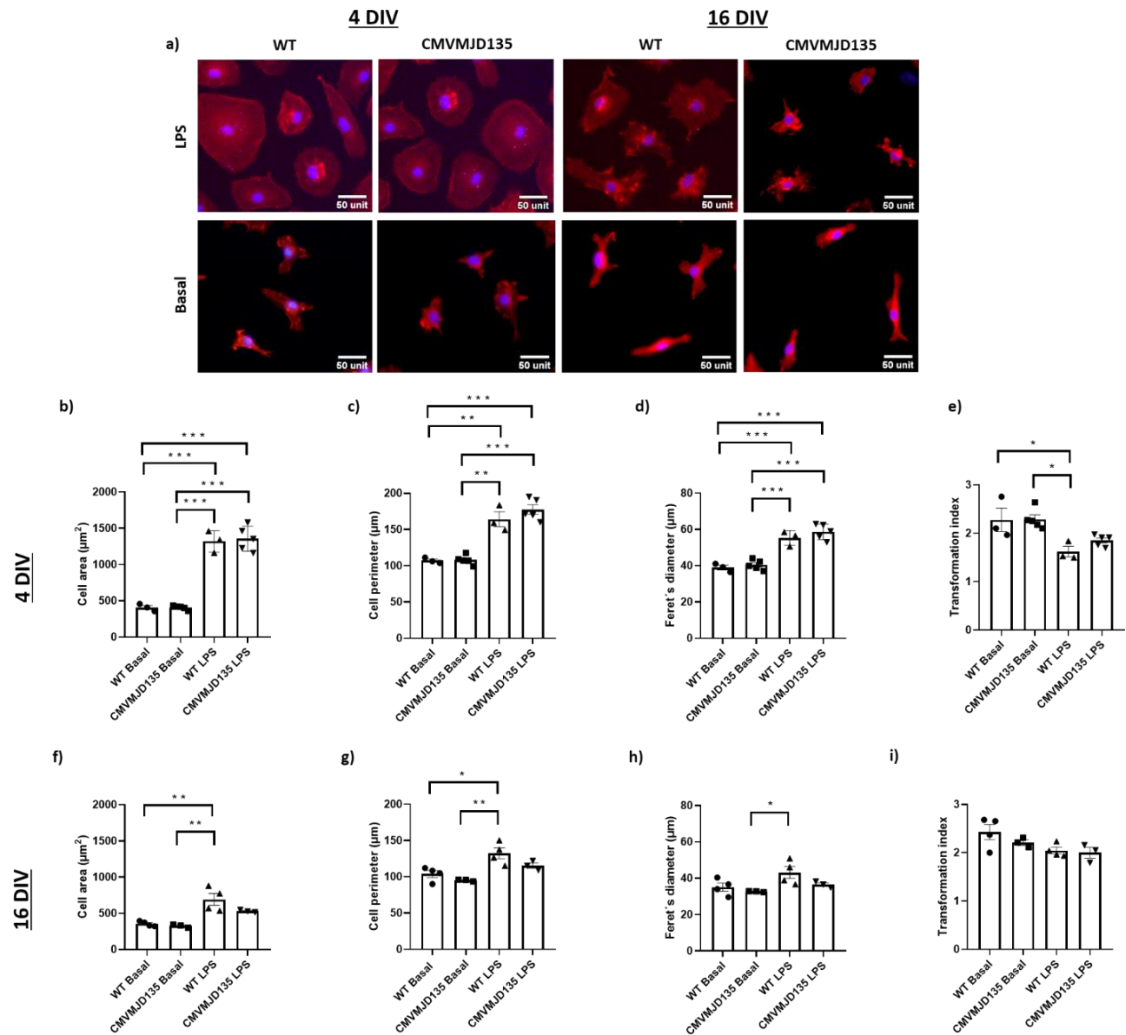
Supplementary Figure S1. Purity assessment of microglia culture over time. (a) Representative images of immunocytochemistry using Iba-1 as a microglial marker (in red) and glial fibrillary acid protein (GFAP) as an astrocyte marker (in green) over time. (b) At 4 days *in vitro* (DIV), a high purity was observed (98%), with a slight contamination with astrocytes occurring over time, but maintaining a 79% purity at 16 DIV. N = 3-4 independent experiments per each time point (4, 10, and 16 DIV). Each value represents the mean \pm SEM. Scale bar: 50 μ m.

Evaluation of the expression levels of mutant human *ATXN3* in microglia



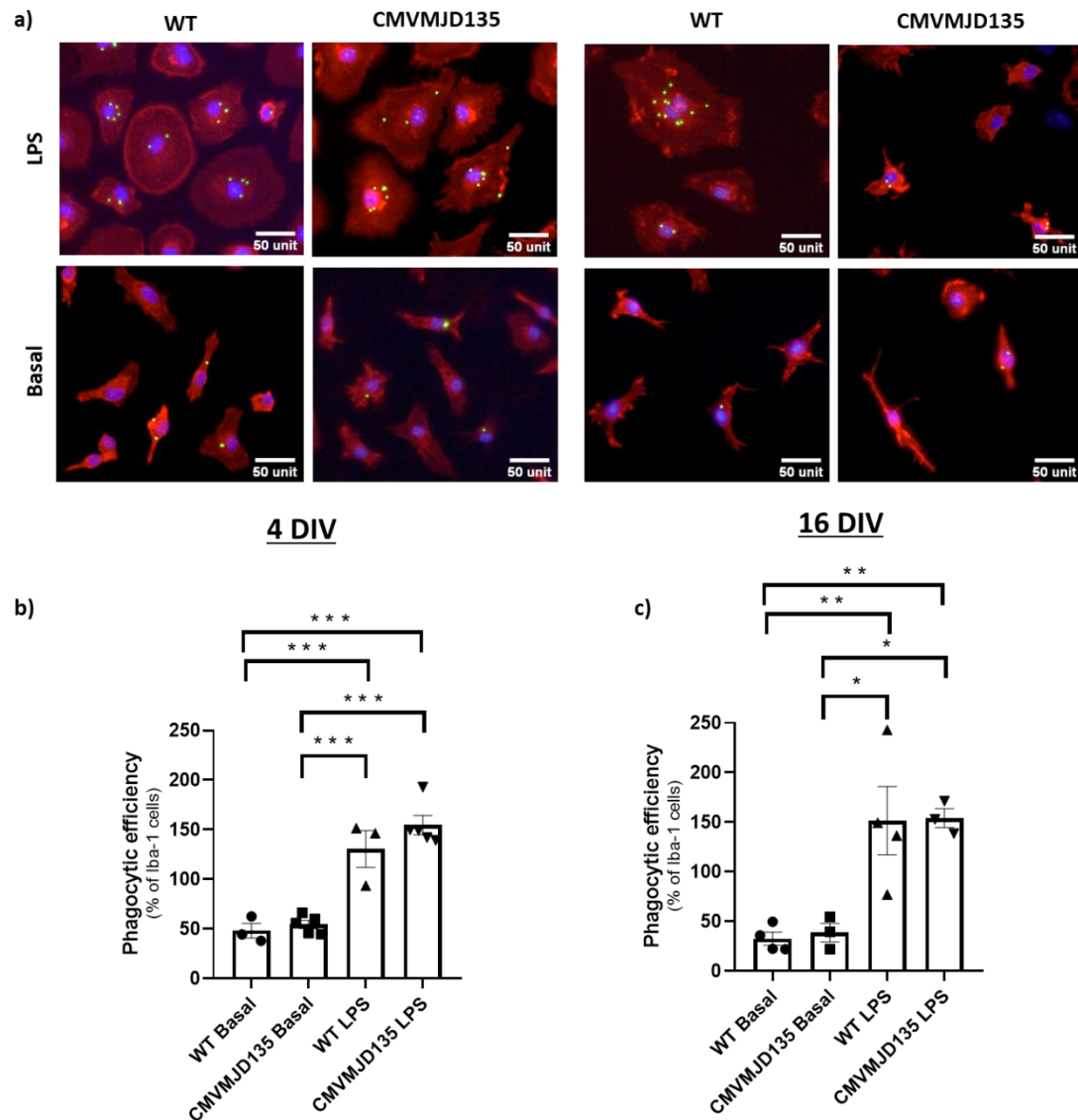
Supplementary Figure S2. Expression of mutant *ATXN3* in microglia from CMVMJD135 mice at two different time points in culture. a) at 4 DIV, and b) at 16 DIV. Cultures of $n = 4-5$ animals per group. Two technical replicates were performed. Fold change ($2^{-\Delta\Delta CT}$) is represented using mouse endogenous *Atxn3* as reference gene. Data are presented as mean+SEM (Student's t-test). *, ***, represent $p < 0.05$ and $p < 0.001$, respectively.

Evaluation of microglial morphology in culture



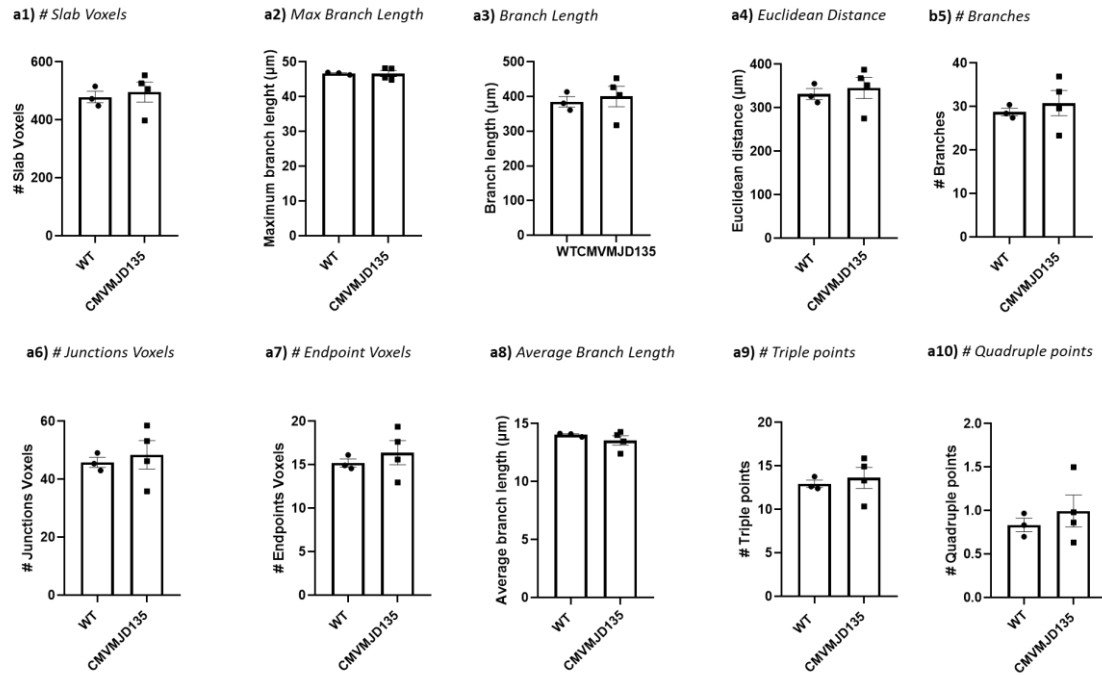
Supplementary Figure S3. Microglia expressing *ATXN3* showed a less activated phenotype in response to lipopolysaccharides (LPS) in artificially “aged” primary cultures. a) Images that represent the morphological changes of microglia, as observed by immunocytochemistry using microglia-specific marker Iba-1 (in red), from CMVMJD135 and Wild-Type (WT) mice, in the absence/presence of LPS, over time, in culture; **b-e)** cell area; **c)** cell perimeter; **d)** Feret's diameter; and **e)** transformation index. **b-e)** measured at 4 DIV; and **f-i)** measured at 16 DIV. Cultures of $n = 3-5$ animals per group. Data are presented as mean+SEM, (One-way ANOVA (Post hoc Tukey's test)). *, **, ***, represent $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Scale bar unit as μm .

Evaluation of microglia phagocytic ability in culture



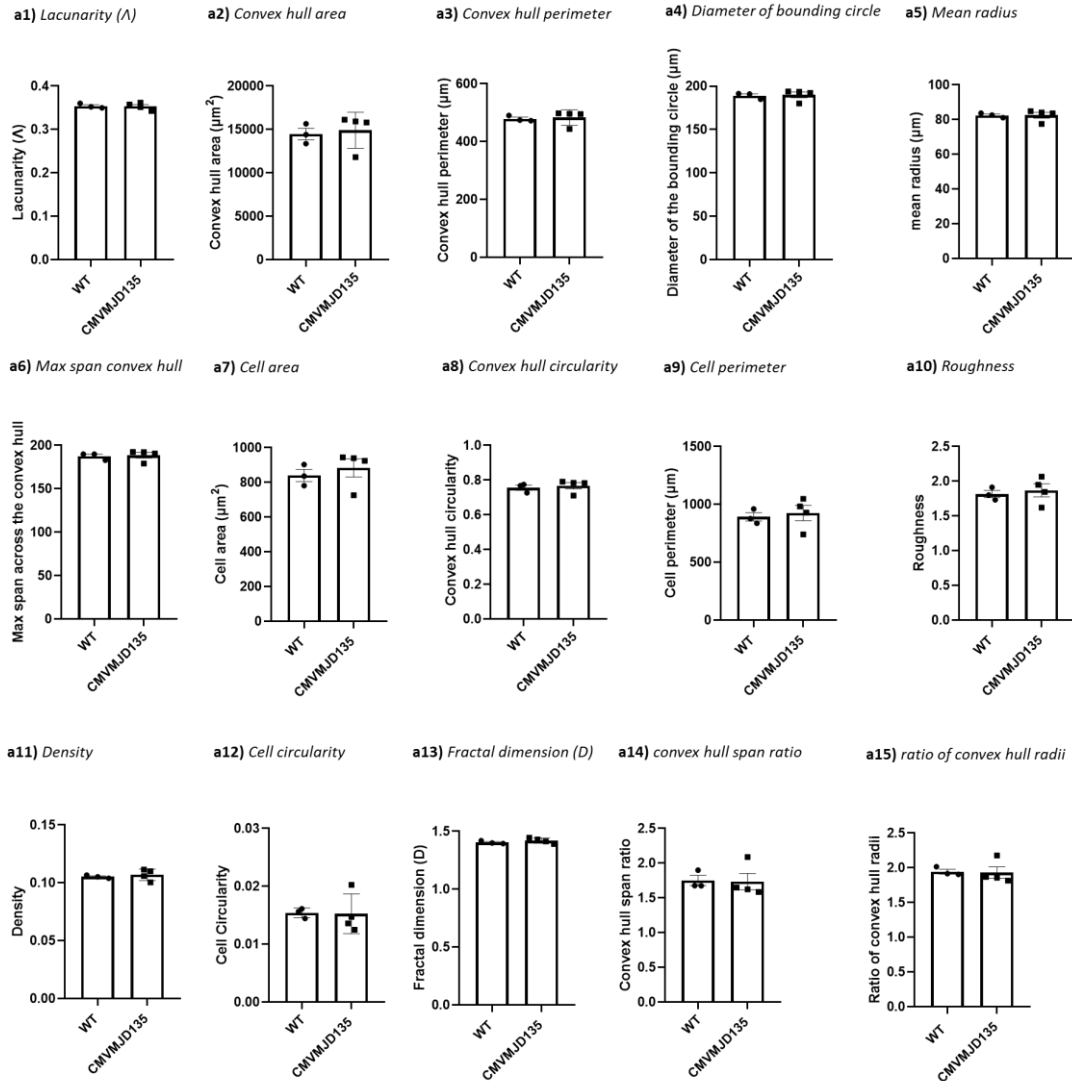
Supplementary Figure S4. CMVMJD135 and WT-derived microglia showed an increased phagocytic efficiency in the presence of LPS in culture. **a)** Representative images of the phagocytic capacity of CMVMJD135 and WT-derived microglia immunostained for Iba-1 (in red) and counterstained with 4',6-Diamidin-2-phenylindol (DAPI) for nuclei staining (in blue) containing phagocytosed fluorescent beads (in green), in the absence/presence of LPS, over time in culture. **b, c)** Phagocytic efficiency (%) was measured using ImageJ and calculated as previously described. Cultures of n = 3-5 per group. Data are presented as mean+SEM, (One-way ANOVA (Post hoc Tukey's test)). *, **, ***, represent $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Scale bar unit as μm .

Morphological analysis of microglia from the PN of CMVMJD135 and WT mice



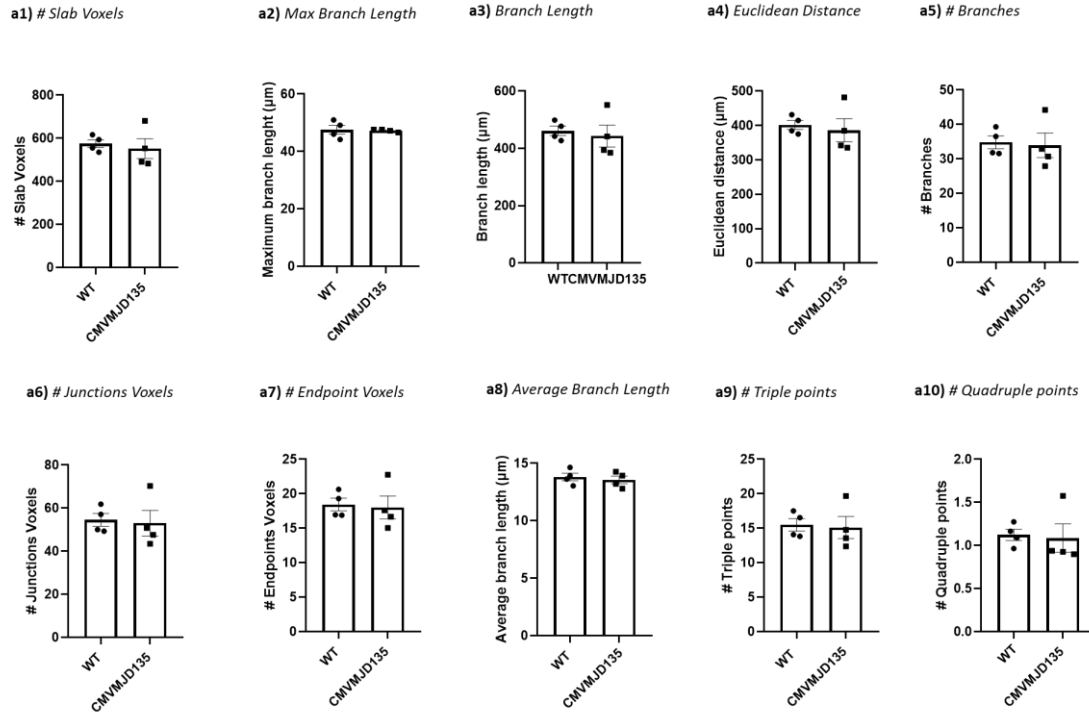
Supplementary Figure S5. The ramification state of microglia in the pontine nuclei (PN) of the CMVMJD135 mice is similar to those of microglia from WT mice. a) Quantification of the morphometric parameters associated with microglia ramification, including: **a1)** # slab voxels; **a2)** maximum branch length; **a3)** branch length; **a4)** Euclidean distance; **a5)** # branches; **a6)** # junctions' voxels; **a7)** # endpoints voxels; **a8)** average branch length; **a9)** # triple points; and **a10)** # quadruple points. Values for all these parameters were obtained from 152 microglial cells from WT mice (n = 3) and 180 microglial cells from CMVMJD135 mice (n = 4) of the PN. Data are presented as mean+SEM (Student's t-test).

Morphological analysis of microglia from the PN of CMVMJD135 and WT mice



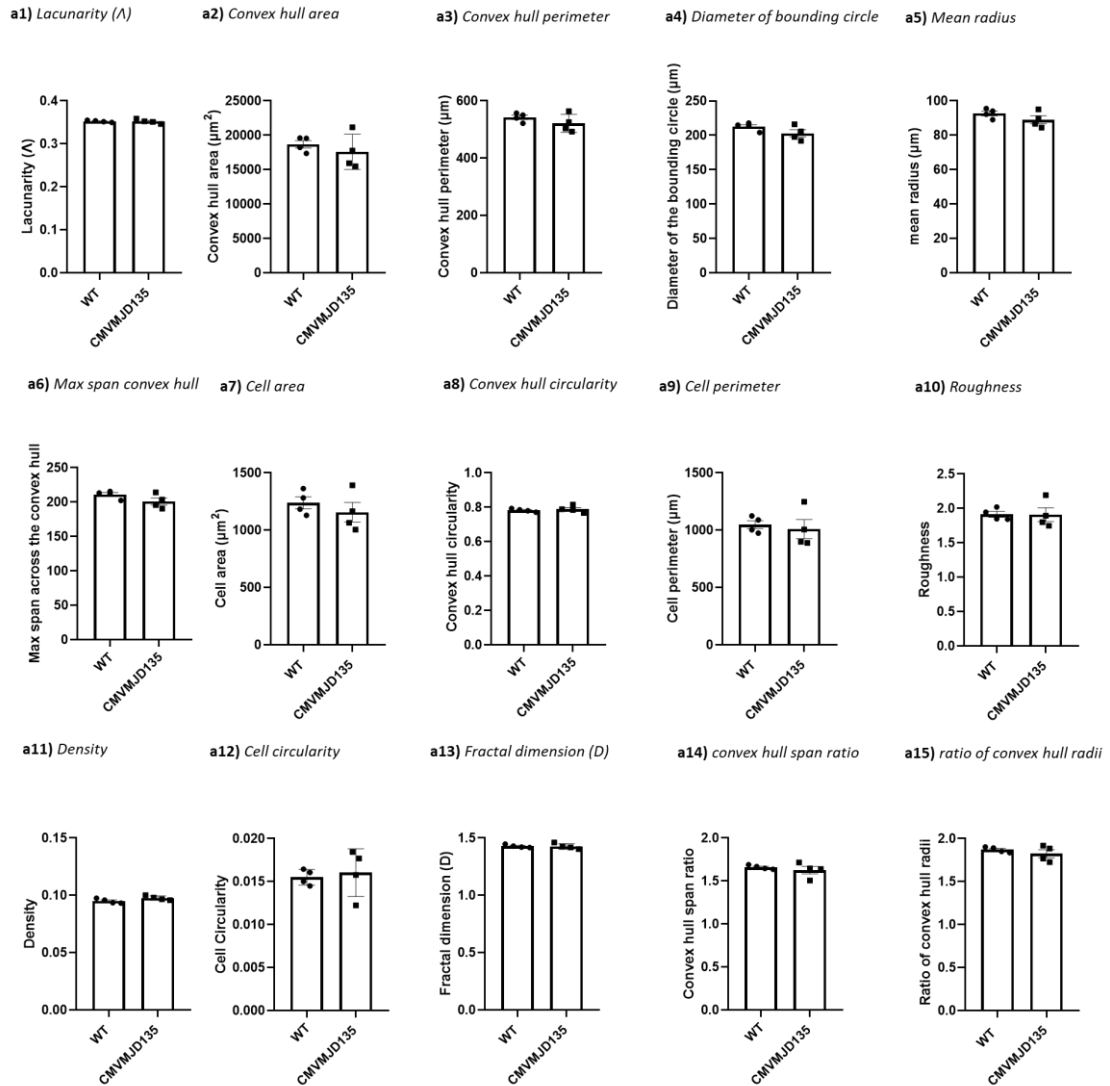
Supplementary Figure S6. The complexity and shape of microglia in the PN of CMVMJD135 mice are similar to those of microglia from WT mice. a) Quantification of the morphometric parameters associated with the heterogeneity of the shape: **a1)** lacunarity (Λ); associated with cell size: **a2)** convex hull area, **a3)** the convex hull perimeter, **a4)** the diameter of bounding circle, **a5)** the mean radius, **a6)** the maximum span across the convex hull, **a7)** the cell area, and **a8)** the convex hull circularity; associated with cell surface **a9)** cell perimeter and **a10)** roughness; associated with soma thickness: **a11)** density and **a12)** cell circularity; associated with the complexity of ramifications: **a13)** fractal dimension (D); and associated with the cylindrical shape of cells: **a14)** convex hull span ratio and **a15)** the ratio of convex hull radii. Values for all these parameters were obtained from 152 microglial cells from WT mice (n = 3) and 180 microglial cells from CMVMJD135 mice (n = 4) of the PN. Data are presented as mean+SEM (Student's t-test).

Morphological analysis of microglia from the DCN of CMVMJD135 and WT mice



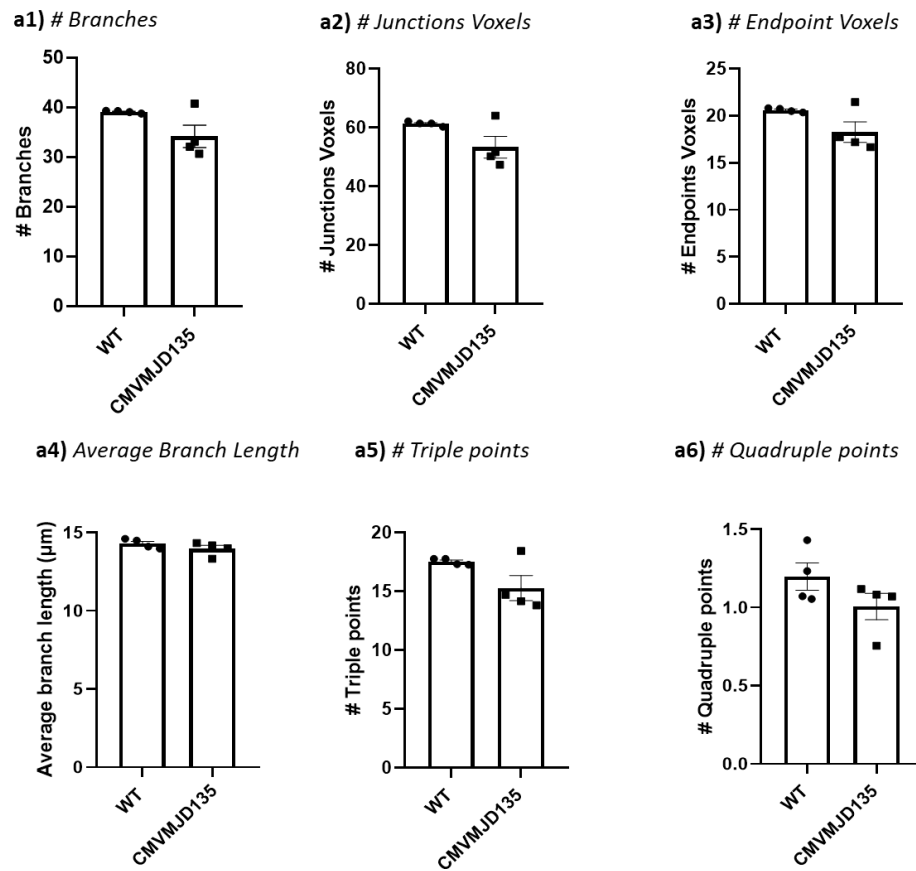
Supplementary Figure S7. Microglia in the deep cerebellar nuclei (DCN) of CMVMJD135 mice showed no differences in features relevant to microglia ramification. a) Quantification of the morphometric parameters associated with microglia ramification including: **a1)** # slab voxels; **a2)** maximum branch length; **a3)** branch length; **a4)** Euclidean distance; **a5)** # branches; **a6)** # junctions' voxels; **a7)** # endpoints voxels; **a8)** average branch length; **a9)** # triple points; and **a10)** # quadruple points. Values for all these parameters were obtained from 349 microglial cells from WT mice (n = 4) and 445 microglial cells from CMVMJD135 mice (n = 4) of the DCN. Data are presented as mean+SEM (Student's t-test).

Morphological analysis of microglia from the DCN of CMVMJD135 and WT mice



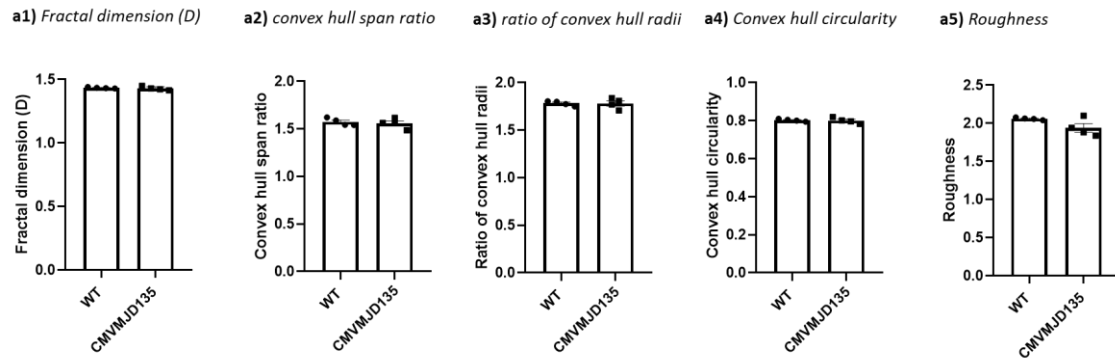
Supplementary Figure S8. Microglia in the DCN of CMVMJD135 mice showed no changes in the complexity and shape. **a)** Quantification of the morphometric parameters associated with heterogeneity of the shape: **a1)** lacunarity (Λ); associated with cell size: **a2)** convex hull area, **a3)** the convex hull perimeter, **a4)** the diameter of bounding circle, **a5)** the mean radius, **a6)** the maximum span across the convex hull, **a7)** the cell area, and **a8)** the convex hull circularity; associated with cell surface: **a9)** cell perimeter and **a10)** roughness; associated with soma thickness: **a11)** density and **a12)** cell circularity; associated with the complexity of ramifications: **a13)** fractal dimension (D); and associated cylindrical shape of the cells: **a14)** convex hull span ratio and **a15)** the ratio of convex hull radii. Values for all these parameters were obtained from 349 microglial cells from WT mice (n = 4) and 445 microglial cells from CMVMJD135 mice (n = 4) of the DCN. Data are presented as mean+SEM (Student's t-test).

Morphological analysis of microglia from the CSC of CMVMJD135 and WT mice



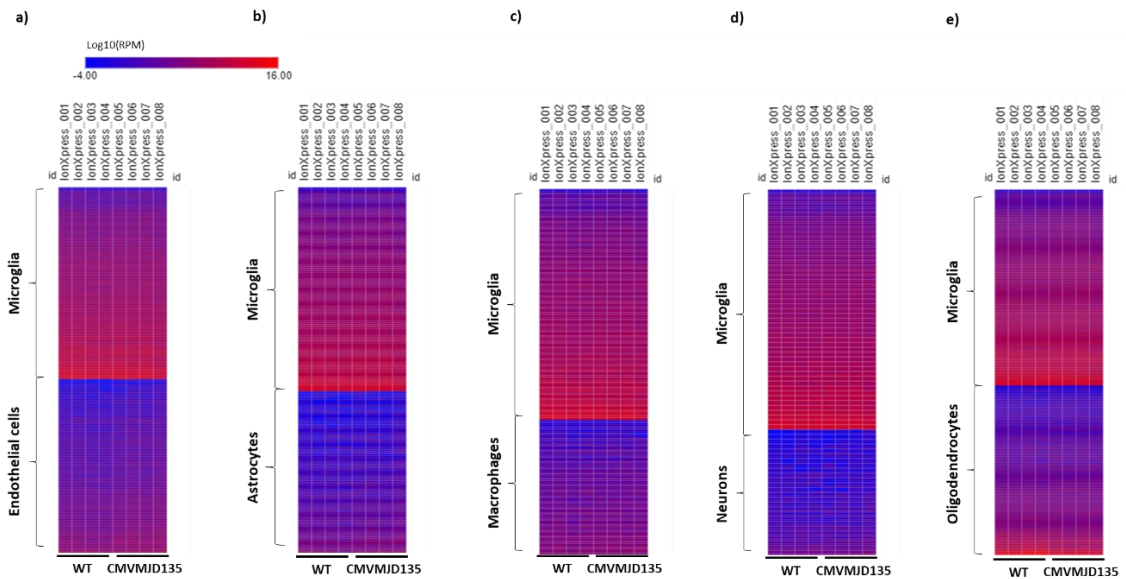
Supplementary Figure S9. Some parameters associated with microglia ramification were similar between CMVMJD135 and WT mice in the cervical spinal cord (CSC). **a)** Quantification of the morphometric parameters associated with microglia ramification including: **a1)** # branches; **a2)** # junctions' voxels; **a3)** # endpoints voxels; **a4)** average branch length; **a5)** # triple points; and **a6)** # quadruple points. Values for all these parameters were obtained from 310 microglial cells from WT mice (n = 4) and 389 microglial cells from CMVMJD135 mice (n = 4) of the CSC. Data are presented as mean+SEM (Student's t-test).

Morphological analysis of microglia from the CSC of CMVMJD135 and WT mice



Supplementary Figure S10. No changes were observed in the parameters related to the complexity of ramifications and with the cylindrical shape of the cells between groups in the CSC. a) Quantification of the morphometric parameters associated with the complexity of ramifications: **a1)** fractal dimension (D); associated with cylindrical shape of the cells: **a2)** convex hull span ratio and **a3)** the ratio of convex hull radii; one of the parameters associated with cell's size: **a4)** the convex hull circularity; and one of the parameters associated with cell's surface: **a5)** roughness. Values for all these parameters were obtained from 310 microglial cells from WT mice (n = 4) and 389 microglial cells from CMVMJD135 mice (n = 4) of the CSC. Data are presented as mean+SEM (Student's t-test).

Evaluation of microglial enrichment in samples from CMVMJD135 and WT mice



Supplementary Figure S11. Evaluation of microglial enrichment in RNA-sequencing samples. Heatmaps showing high levels of expression for specific markers of microglia when compared with markers of other cell-types. a) Microglia versus endothelial cells; b) microglia versus astrocytes; c) microglia versus macrophages; d) microglia versus neurons; and e) microglia versus oligodendrocytes. Four biological replicates for WT and CMVMJD135 mice were used. An heatmap containing the cell-specific markers was achieved using the Clue Morphheus software.

RNA-sequencing analysis of microglia from CMVMJD135 and WT mice

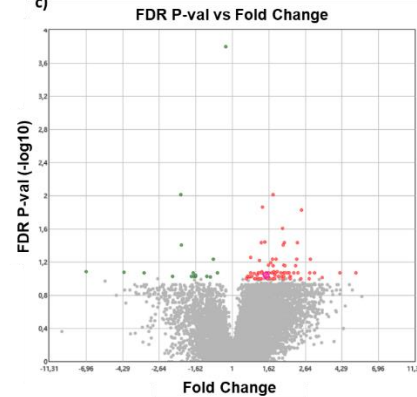
a)

ID	Fold Chan...	P-val	FDR P-val	Public Gene IDs	Gene Symbol
count: 83					
Ufi1	1,72	1,63E-06	0,0097	NM_026194	Ufi1
Lrrc58	1,49	3,44E-06	0,0137	NM_177093	Lrrc58
Lamc1	2,5	4,34E-06	0,0149	NM_010683	Lamc1
Cdyl	1,95	8,26E-06	0,0247	NM_001123386	Cdyl
Rnf144b	1,54	1,37E-05	0,0364	NM_001170643	Rnf144b
Ncam1	2	1,55E-05	0,0367	NM_001081445	Ncam1
Ddb1	1,47	1,75E-05	0,0367	NM_015735	Ddb1
Cux2	2,36	1,84E-05	0,0367	NM_001312908	Cux2
A2m	1,97	2,29E-05	0,0391	NM_175628	A2m
Epsti1	1,28	3,46E-05	0,0552	NM_029495	Epsti1
Hipk3	1,71	3,88E-05	0,0581	NM_001145824	Hipk3
Ccdc151	2,36	4,16E-05	0,0581	NM_001163787	Ccdc151
Arhgef12	1,79	4,49E-05	0,0581	NM_027144	Arhgef12
Pcdhb21	2,83	4,77E-05	0,0581	NM_053146	Pcdhb21
Mef2c	1,43	5,24E-05	0,0597	NM_001170537	Mef2c
Mira	1,67	5,88E-05	0,0639	NR_045199	Mira
L3mbt13	1,98	6,73E-05	0,0686	NM_172787	L3mbt13
Fam188b	1,62	6,88E-05	0,0686	NM_001142781	Fam188b
Gm6548	2,33	7,64E-05	0,0697	NR_003363	Gm6548
Tmem136	2,02	7,78E-05	0,0697	NM_001034863	Tmem136
4930564C03Rik	1,72	7,86E-05	0,0697	4930564C03Rik	
Pcnx3	1,82	0,0001	0,0817	NM_144868	Pcnx3
Scd2	1,74	0,0001	0,0817	NM_009128	Scd2
Haghl	1,48	0,0001	0,0817	NM_001271433	Haghl
Zfp568	2,01	0,0001	0,0850	NM_001033355	Zfp568
Ak8	1,98	0,0001	0,0850	NM_001033874	Ak8
Mpg	1,69	0,0001	0,0850	NM_010822	Mpg
Ap3m2	1,61	0,0001	0,0850	NM_001122820	Ap3m2
Sox8	2,07	0,0001	0,0850	NM_011447	Sox8
Rbbp6	1,56	0,0001	0,0850	NM_011247	Rbbp6

b)

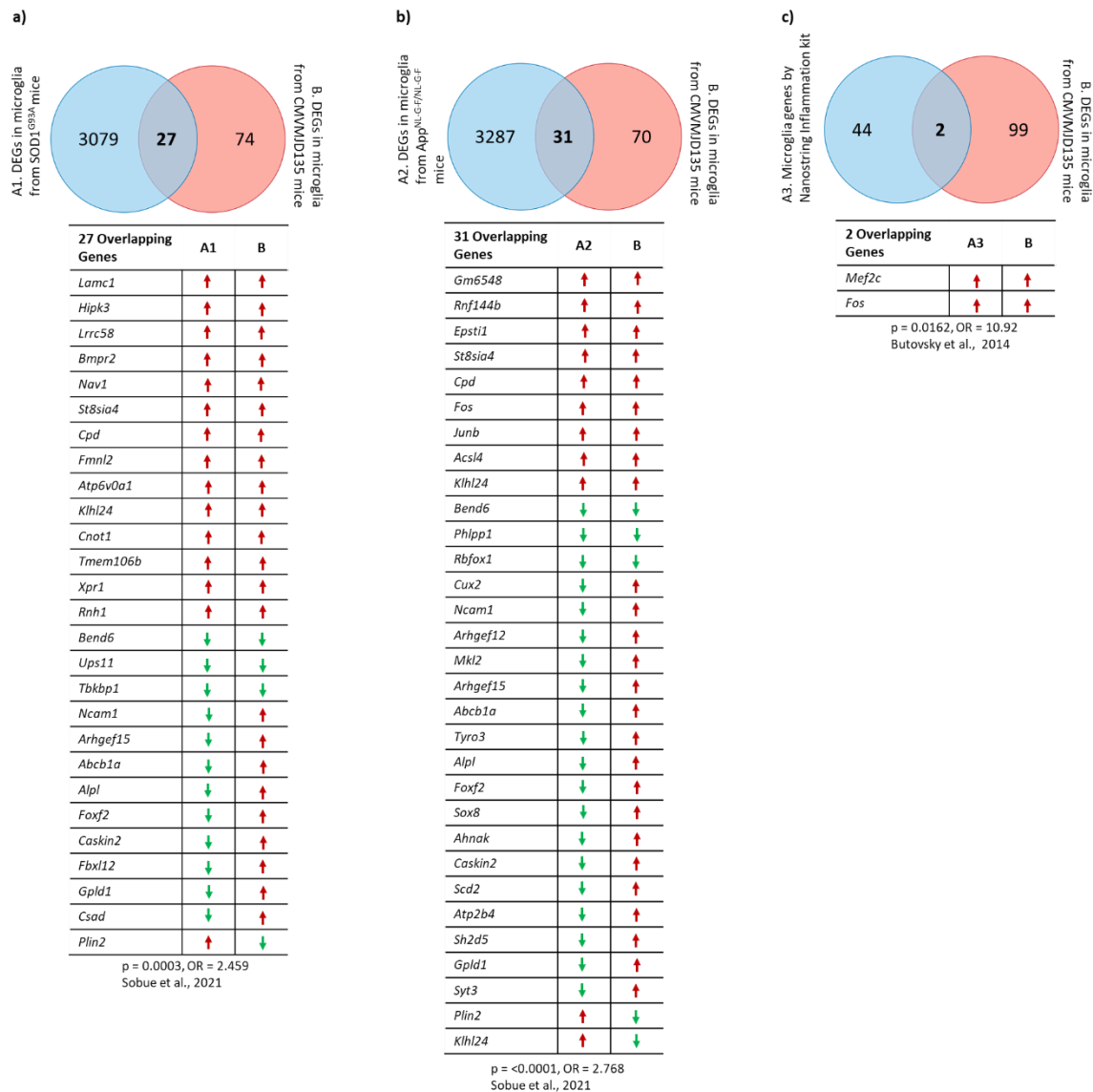
ID	Fold Chan...	P-val	FDR P-val	Public Gene IDs	Gene Symbol
count: 18					
4921530L21Rik	-1,09	1,98E-08	0,0002	NM_025733	4921530L21Rik
Cpsf1	-1,09	1,98E-08	0,0002	NM_001164173	Cpsf1
Olfir791	-1,09	1,98E-08	0,0002	NM_146930	Olfir791
Zfp62	-1,98	2,04E-06	0,0097	NM_001024846	Zfp62
Bend6	-1,97	2,16E-05	0,0391	NM_001310484	Bend6
Usp11	-1,28	4,85E-05	0,0581	NM_145628	Usp11
Gm2694	-6,97	9,62E-05	0,0817	NR_033430	Gm2694
LOC108169012	-4,19	0,0001	0,0841	XR_001784563	LOC108169012
Akap7	-3,22	0,0001	0,0850	NM_018747	Akap7
Fbxw4	-1,68	0,0002	0,0850	NM_013907	Fbxw4
Nek9	-1,22	0,0002	0,0850	NM_145138	Nek9
Hoxb8	-1,63	0,0002	0,0906	NM_010461	Hoxb8
Phlpp1	-1,67	0,0003	0,0930	NM_133821	Phlpp1
Plin2	-1,41	0,0003	0,0930	NM_007408	Plin2
Pnpla3	-1,63	0,0003	0,0930	NM_054088	Pnpla3
Rbfox1	-2,21	0,0003	0,0930	NM_021477	Rbfox1
Tasp1	-1,72	0,0003	0,0930	NM_001159640	Tasp1
Tbkbp1	-1,34	0,0003	0,0954	NM_198100	Tbkbp1

c)



Supplementary Figure S12. Differential gene expression between microglial cells from CMVMJD135 and WT mice. a, b, c) Up-regulated and down-regulated genes were determined using the Transcriptome Analysis Console (TAC) software, between CMVMJD135 and WT mice. a) Heatmap of 83 up-regulated genes in microglial cells from CMVMJD135 mice, in ascending order of False Discovery Rate (FDR) value. b) Heatmap of 18 down-regulated genes in microglial cells from CMVMJD135 mice, in ascending order of FDR value. c) Volcano plot view of CMVMJD135 versus WT genes. Red for up-regulated genes and green for down-regulated genes. |fold change| > 1, p < 0.05, and an FDR < 0.1 was considered to determine genes significantly differentially expressed.

Transcriptional changes seen in CMVMJD135 microglia overlap those in Amyotrophic lateral sclerosis (ALS) and Alzheimer disease (AD) mouse models



Supplementary Figure S13. Transcriptional changes seen in CMVMJD135 microglia overlap those in Amyotrophic lateral sclerosis (ALS) and Alzheimer disease (AD) mouse models. Venn diagrams and table overview representing the overlapping genes between the 101 CMVMJD135-altered genes found in our RNA-sequencing analysis, with **a)** 3106 differentially expressed genes (DEGs) previously reported in the microglia of a mouse model of ALS, SOD1^{G93A} mouse [51]; with **b)** 3318 DEGs previously reported in the microglia of a mouse model of AD, App^{NL-G-F/NL-G-F} mouse [51]; and with **c)** 46 microglial genes highly expressed and/or affected in microglia in different neuroinflammatory conditions, as detected by the Nanostring inflammation kit [64]. Red arrows represent the up-regulated genes and green arrows represent the down-regulated genes. Comparisons were conducted by contingency analysis, using the Fisher's exact test and the Baptista-Pike method to calculate the odds-ratio. Significance was set at p < 0.05.

2. Supplementary References

[51] Sobue, A.; Komine, O.; Hara, Y.; Endo, F.; Mizoguchi, H.; Watanabe, S.; Murayama, S.; Saito, T.; Saido, T.C.; Sahara, N.; Higuchi, M.; Ogi, T.; Yamanaka, K. Microglial gene signature reveals loss of homeostatic microglia associated with neurodegeneration of Alzheimer's disease. *Acta Neuropathologica Communications* 2021, 9. doi:10.1186/s40478-020-01099-x.

[64] Butovsky, O.; Jedrychowski, M.P.; Moore, C.S.; Cialic, R.; Lanser, A.J.; Gabriely, G.; Koeglspenger, T.; Dake, B.; Wu, P.M.; Doykan, C.E.; Fanek, Z.; Liu, L.; Chen, Z.; Rothstein, J.D.; Ransohoff, R.M.; Gygi, S.P.; Antel, J.P.; Weiner, H.L. Identification of a unique TGF- β -dependent molecular and functional signature in microglia. *Nature Neuroscience* 2014, 17, 131–43. doi:10.1038/nn.3599.