**Supplementary Information to**

**TREM2 triggers microglial density and age-related neuronal loss**

***Running title:* TREM2 contributes to the aging process**

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**Methods**

***RNA sequencing.*** Twelve samples were prepared: 6 TREM2 wt and 6 TREM2 KOs. Sequencing library preparation was done with 1 µg of total RNA using the TruSeq mRNA Stranded Library Prep Kit (Illumina, USA) according to manufacturer’s protocol. Briefly, the mRNA pull down was done using the magnetic beads with oligo-dT primer. Fragmented RNA was reverse transcribed, and second strand synthesis was done with incorporation of dUTP so that during PCR amplification only first strand was amplified. The libraries were quantified using Qubit dsDNA HS assay kit (Thermo Fisher Scientific, USA) and the quality was determined using 2100 Bioanalyzer (Agilent, USA). Pooled libraries were sequenced on NextSeq500 using manufacturer’s instructions. After sequencing, reads were processed on the High Performance Computer of the University of Luxembourg (Varrette, Bouvry, Cartiaux, & Georgatos, 2014). Reads quality was assessed using FastQC (v0.11 http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Due to a slight loss of base qualities observed at the read ends, reads were trimmed using AdapterRemoval [v2.2 (Schubert, Lindgreen, & Orlando, 2016)]. Genome indexing was done with the mouse reference *mm10* (GRCm38.p3) with the ensembl gene annotation version 79 for the STAR aligner [v2.5.2b (Dobin et al., 2013)], setting the option --sjdbOverhang to 78. Mapping was then performed with the following command, using tweaked options suggested by Barruzo *et al*. (Baruzzo et al., 2017): STAR --twopassMode Basic --outSAMunmapped Within --limitOutSJcollapsed 1000000 --limitSjdbInsertNsj 1000000 --outFilterMultimapNmax 100 --outFilterMismatchNmax 33 --outFilterMismatchNoverLmax 0.3 --seedSearchStartLmax 12 --alignSJoverhangMin 15 --alignEndsType Local --outFilterMatchNminOverLread 0 --outFilterScoreMinOverLread 0.3 --winAnchorMultimapNmax 50 --alignSJDBoverhangMin 3 --outFilterType BySJout --outSAMtype BAM SortedByCoordinate (mapping statistics provided by the STAR log files see supplementary table 1).

Transcript counts were obtained from the BAM files by the R package *Rsubread* [v1.26 (Liao, Smyth, & Shi, 2013)] using R [v3.3 (R Core Team, 2017)]. Next, computation and plotting were done with R and Rstudio (RStudio Team, 2016). The differential expression analysis was performed by the R package *DESeq2* [v1.15 (Love, Huber, & Anders, 2014)] with the contrast wt versus TREM2 KO. Transcript annotations were retrieved from ensembl (archive from March 2015) with the R bioconductor package *biomaRt* [v2.34 (Durinck, Spellman, Birney, & Huber, 2009)].

Plots were done using the R package *ggplot2* [v2.1 (Wickham, 2009)] and the collection of R packages *tidyverse* [v1.1 (Wickham, 2017)]. Raw FASTQ and BAM files have been deposited in the European Nucleotide Archive (ENA accession number: PRJEB23660).

***Pathway enrichment analysis.*** For Ingenuity Pathway Analysis (IPA), list of differentially expressed genes (log2FC>0.5, FDR<0.05) in TREM2 KO mice compared to the wt mice were uploaded in the IPA tool (Ingenuity Systems, www.ingenuity.com). The significance of the association between each list and function or canonical pathway was measured by Fisher’s exact test.

***Oligonucleotides for sqRT PCR.*** *18S* forward (for) – CTCAACACGGGAAACCTCAC; *18S* reverse (rev) – CGCTCCACCAACTAAGAACG (Lin et al., 2013); *Aif1* for – GAAGCGAATGCTGGAGAAAC; *Aif1* rev – AAGATGGCAGATCTCTTGCC; *beta-Actin* for – GGCTGTATTCCCCTCCATCG; *beta-Actin* rev – CCAGTTGGTAACAATGCCATGT (Ruiz-Villalba et al., 2017); *C1qa* for – AGAGGGGAGCCAGGAGC; *C1qa* rev – CTTTCACGCCCTTCAGTCCT; *C1qb* for – GACTTCCGCTTTCTGAGGACA; *C1qb* rev – CAGGGGCTTCCTGTGTATGGA; *C1qc* for - GCCTGAAGTCCCTTACACCC; *C1qc* rev – GGGATTCCTGGCTCTCCCTT; *C3* for – TAGTGCTACTGCTGCTGTTGGC; *C3* rev – GCTGGAATCTTGATGGAGACGCTT (Linnartz, Kopatz, Tenner, & Neumann, 2012); *C4b* for – TGGAGGACAAGGACGGCTA; *C4b* rev – GGCCCTAACCCTGAGCTGA (Haga, Aizawa, Ishii, & Ikeda, 1996); *Cd68* for – CAGGGAGGTTGTGACGGTAC; *Cd68* rev – GAAACATGGCCCGAAGTATC; *Cyba* for – CCTCCACTTCCTGTTGTCGG; *Cyba* rev – TCACTCGGCTTCTTTCGGAC; *Cybb* for – GGGAACTGGGCTGTGAATGA; *Cybb* rev – CAGTGCTGACCCAAGGAGTT; *Dap12* for – ATGGGGGCTCTGGAGCCCT; *Dap12* rev – TCATCTGTAATATTGCCTCTGTGT; *Fcer1g* for – CTGTCTACACGGGCCTGAAC; *Fcer1g* rev – AAAGAATGCAGCCAAGCACG; *Gapdh* for – AACTTTGGCATTGTGGAAGG; *Gapdh* rev – GGATGCAGGGATGATGTTCT; *Il-1β* for – CTTCCTTGTGCAAGTGTCTG; *Il-1β* rev – CAGGTCATTCTCATCACTGTC; *Inos* for – AAGCCCCGCTACTACTCCAT; *Inos* rev – GCTTCAGGTTCCTGATCCAA; *Itgam* for – CATCAAGGGCAGCCAGATTG; *Itgam* rev – GAGGCAAGGGACACACTGAC; *Itgb2* for – GTCCCAGGAATGCACCAAGT; *Itgb2* rev – CCGTTGGTCGAACTCAGGAT; *Rpl13a* for – TGGTCCCTGCTGCTCTCA; *Rpl13a* rev – CCCCAGGTAAGCAAACTTTCT (Bouvy-Liivrand et al., 2014); *Tmem119* for - GTGTCTAACAGGCCCCAGAA; *Tmem119* rev – AGCCACGTGGTATCAAGGAG (Bennett et al., 2016); *Tnfα* for – GGTGCCTATGTCTCAGCCTC; *Tnfα* rev – TGAGGGTCTGGGCCATAGAA.

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**Supplementary table**

Supplementary table 1: Mapping statistics using the STAR aligner (according to STAR statistics)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| sample | # input reads | # uniquely mapped reads | uniquely mapped reads (%) | average length (bp) |
| KO01 | 39,300,660 | 36,954,171 | 94.03 | 77.85 |
| KO02 | 40,106,764 | 37,800,647 | 94.25 | 77.63 |
| KO03 | 43,838,950 | 41,176,165 | 93.93 | 77.38 |
| KO04 | 37,475,817 | 35,234,853 | 94.02 | 77.79 |
| KO05 | 40,037,054 | 37,717,598 | 94.21 | 77.96 |
| KO06 | 38,943,357 | 36,666,640 | 94.15 | 77.79 |
| WT01 | 38,993,187 | 36,707,258 | 94.14 | 78.04 |
| WT02 | 38,378,217 | 36,030,252 | 93.88 | 77.94 |
| WT03 | 34,722,079 | 32,635,368 | 93.99 | 77.26 |
| WT04 | 38,172,961 | 35,963,936 | 94.21 | 78 |
| WT05 | 40,225,273 | 37,903,713 | 94.23 | 77.79 |
| WT06 | 42,881,496 | 40,336,779 | 94.07 | 78 |
| Total | 473,075,815 | 445,127,380 | NA | NA |

**Supplementary Figures**

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**Figure S1:** **Comparison of housekeeping gene transcripts between TREM2 KO mice and littermate controls. A** Vulcano plot showing all genes (each dot respresents a transcript; (log2FoldChange (FC) versus –log10[adjusted p-value])) as identified by RNA-seq analysis in half brain samples of TREM2 KO mice. Genes with abs(log2FC)≥0.5 and adjusted p-value (padj)<0.05 are highlighted in orange. Moreover, the three housekeeping genes Gapdh, beta-Actin (Actb) and Rpl13a are labelled in red, showing that they are very stable between the two conditions (Gapdh: padj=0.87; Actb: padj=0.73; Rpl13a: padj=0.99). n=6 mice per group. **B** Semi-quantitative real-time (sqRT) PCR of the housekeeping genes *Gapdh, beta-Actin, 18S* and *Rpl13a* in half brains of 24 months old TREM2 wt and KO mice. None of the investigated housekeeping genes shows differences in the cycle threshold in-between TREM2 wt and KO mice. Data are presented as mean+s.e.m. n=5-6 mice per group. n.s. not significant.

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**Figure S2: Less age-related neuronal loss in TREM2 KO mice.** Quantification of the width of hippocampal dentate gyrus (DG, *left*) and CA3 (*right*) of 3, 12 and 24 months old TREM2 wildtype (wt) versus knock-out (KO) mice showed an increased DG and CA3 width in 24 months old TREM2 KO mice. n=6-12 mice per group. Data are presented as mean±s.e.m. Values were normalized to 3 months old wt animals. Multiple linear regression model with post hoc LSD; \* p≤0.05; \*\*\* p≤0.001; n.s. not significant.

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**Figure S3: Genes, molecules and pathways differentially regulated in aged TREM2 KO mice. A** Plot demonstrates the 211 differentially expressed (DE) genes (log2FC>0.5, FDR<0.05) identified by RNA-seq analysis in half brain samples of TREM2 KO mice that were used for Ingenuity Pathway Analysis (IPA) in panels B-D. The genes are listed according to their fold change in TREM2 KO mice. Genes of particular interest are highlighted. The bars indicate the fold change in log2-scale and circles are color-coded according to statistical significance of the expression change (adjusted p-value, Benjamini-Hochberg correction). n=6 mice per group. **B** The top six canonical pathways with highest enrichment for the DE genes in TREM2 KO include the complement system and the production of nitric oxide and reactive oxygen species in macrophages. **C** Among the top five molecular and cellular functions as revealed by IPA are cell-to-cell signaling and interaction, and cellular function and maintenance. **D** The top six upstream regulators predicted by IPA to control DE genes in TREM2 KO are ifnγ, tnfα, il4, il-1ß, il6 and ifnα. The predicted mode of regulation is inhibition. The significance of the enrichments was derived using Fisher´s exact test and are shown as -log(p-value).