**METHODS**

**RNASeq Quality check and alignment**

RNASeq fastq files were analyzed with FastQC[1] v0.11.7 to assess sequence base quality, per-base sequence content, GC content, N content, and the sequence length distribution. Reads were subsequently trimmed using Trimmomatic[2] v0.38, to remove Illumina adapters, leading and trailing bases with score ≤ 3, all bases after the sliding window average ≤ 15, and all edited reads ≤ 36 bp. Reads were aligned to *Mus musculus* annotation GRCm38.p6 using Salmon[3] v0.10.0. Default parameters were used for building the mouse index, and for alignment of transcripts.

**Differential expression analysis**

DESeq2[4] v1.18.1 was used to assess differential gene expression using the likelihood ratio test, with the model

~ replicate + condition

analyzed against the reduced model

~ replicate

Variance Stabilized Transformed gene counts were used to identify outliers, using both Principal Component Analysis and sample clustering (using the euclidean distance metric and the complete clustering method). Two samples - wildtype replicate C and knockout 2 replicate A - were removed from further analysis.

To identify significant contrasts between treatments, a *post hoc* analysis of genes differentially expressed according to LRT analysis was performed. DESeq2 was used to perform the nbinomial Wald test for contrasts between PBS, WT, KO1 and KO2.

The focal gene set was identified as those genes in which:

* the likelihood ratio test was significant (*p* ≤ 0.05)
* WT and PBS were significantly differentially expressed (*p* ≤ 0.05)
* WT was significantly different from both KO1 and KO2 (*p* ≤ 0.05 in each contrast)
* KO1 and KO2 were concordantly up- or down- regulated with regard to WT

Log2(Fold change) values and *p* values are reported according to the Wald tests.

Gene names were mapped to entrez gene identifiers using ensembl biomart[5], mouse version GRCm38.p6.

All scripts and output of the differential analysis are available at doi: 10.5281/zenodo.2574121. The R Script used for differential expression analysis can be found in supplementary file differential-expression-analysis.R The python script used for subsequent post hoc analysis can be found in the Jupyter notebooks endothelial\_expression\_analysis.ipynb and microglial\_expression\_analysis.ipynb.

**Bibliography**

1. Andrews, S. FastQC: a quality control tool for high throughput sequence data. (2010). at <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>
2. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120 (2014).
3. Patro, R., Duggal, G., Love, M. I., Irizarry, R. A. & Kingsford, C. Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods 14, 417–419 (2017).
4. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550 (2014).
5. Zerbino, D. R. et al. Ensembl 2018. Nucleic Acids Res. 46, D754–D761 (2018).