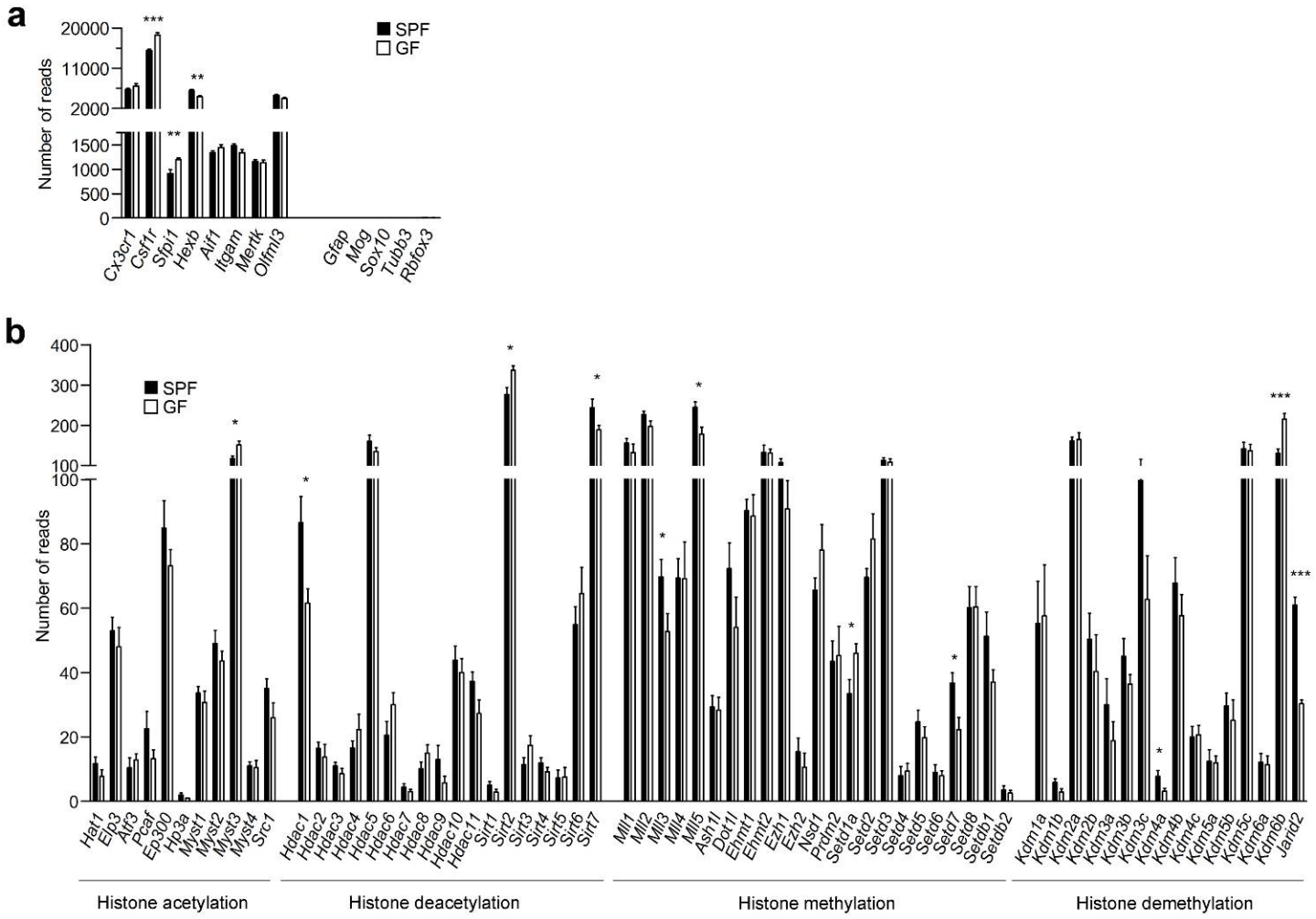
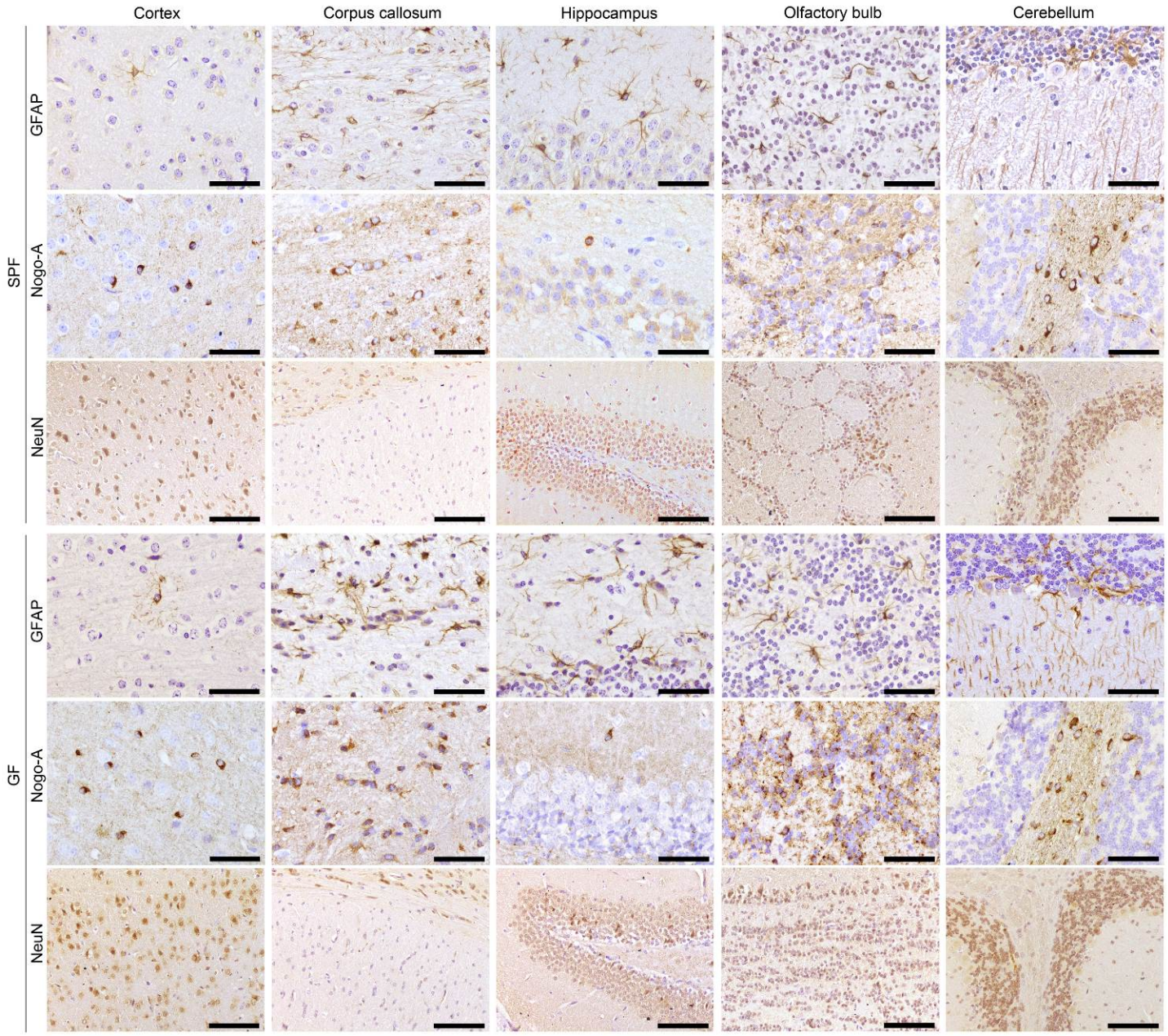



**Supplementary Figure 1**

Gene profile from microglia in GF mice.

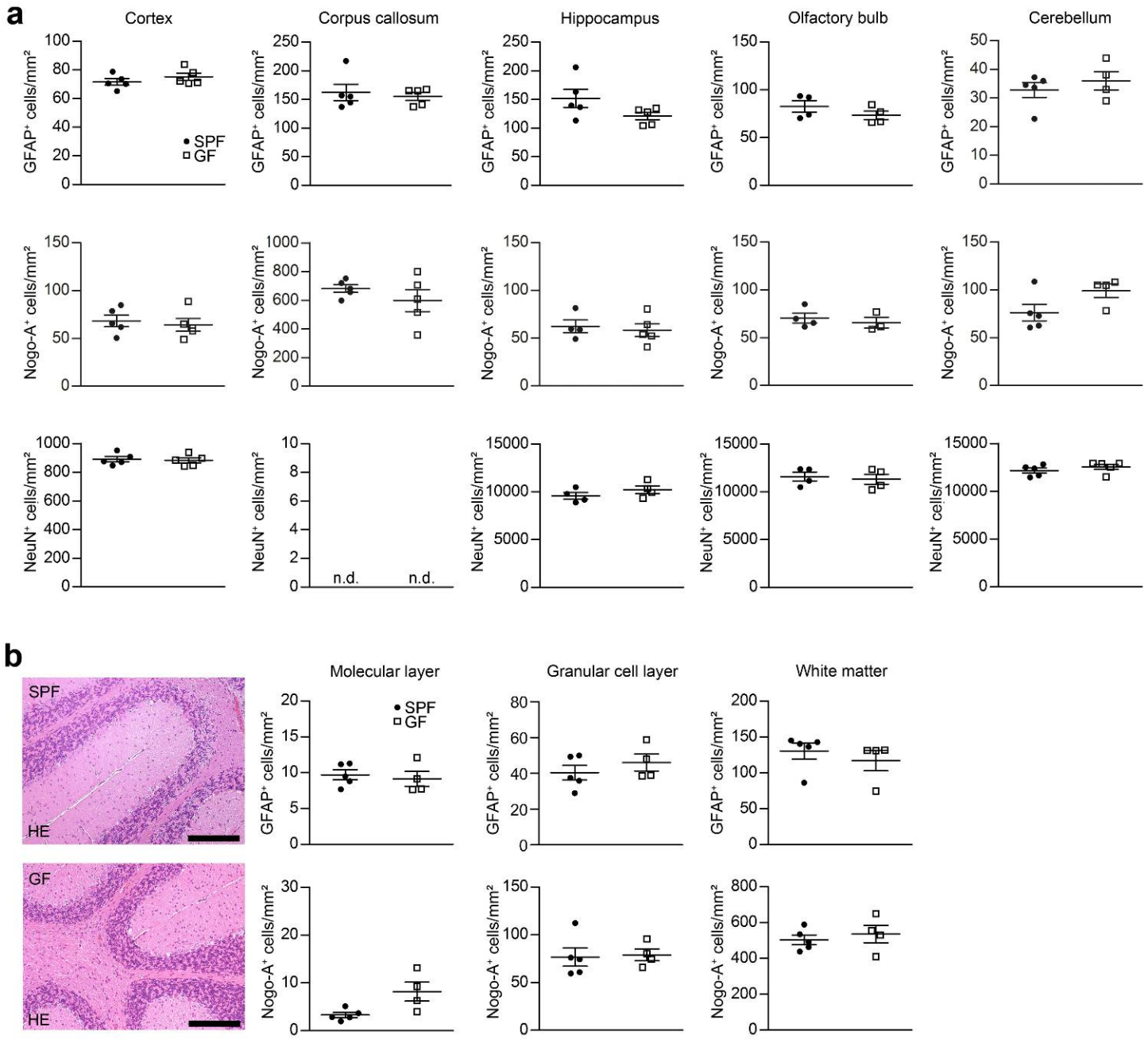
mRNA expression values (number of reads) of genes from microglia in GF animals (white bars) or SPF mice (black bars). **(a)** Prototypical microglia genes are presented and compared to the non-microglia genes such as *Gfap, Mog, Tubb3* and *Rbfox3*. **(b)** Epigenetic signature of microglia was examined by investigating genes (number of reads) related to histone acetylation, deacetylation, methylation and demethylation, respectively. Bars represent means ± s.e.m. with seven samples in each group. Significant differences were determined by an unpaired *t* test and marked with asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). *P* values: *Csf1r*: 0.0001, *Sfpi1*: 0.0075, *Hexb*: 0.0060. *Myst3*: 0.0121, *Hdac1*: 0.0191, *Sirt2*: 0.0113, *Sirt7*: 0.0441, *Mll3*: 0.0489, *Mll5*: 0.0105, *Setd1a*: 0.0360, *Setd7*: 0.0134, *Kdm4a*: 0.0476, *Kdm6b*: 0.0005, *Jarid2*: <0.0001.




**Supplementary Figure 2**

Absence of microbes does not alter the amount of neurons, astrocytes and oligodendrocytes.

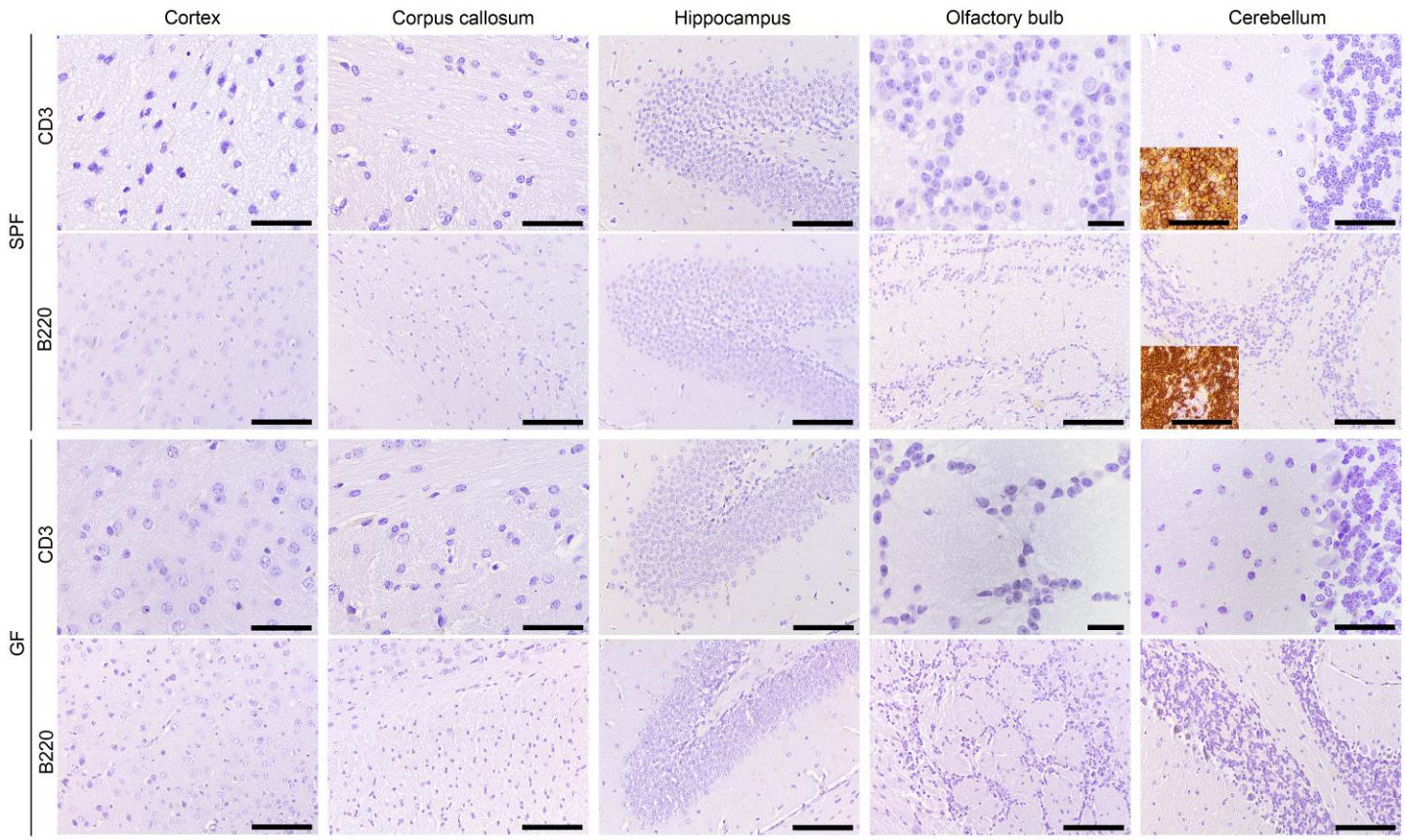
CNS histology of the neuroectodermal compartment of 6-8 weeks old GF or control SPF mice. NeuN labels mature neurons, Nogo-A oligodendrocytes and glial fibrillary acidic protein (GFAP) astrocytes. Representative pictures are shown. Scale bars: NeuN: 100 µm, Nogo-A and GFAP: 50 µm.




**Supplementary Figure 3**

Absence of microbes does not alter the amount of neurons, astrocytes and oligodendrocytes.

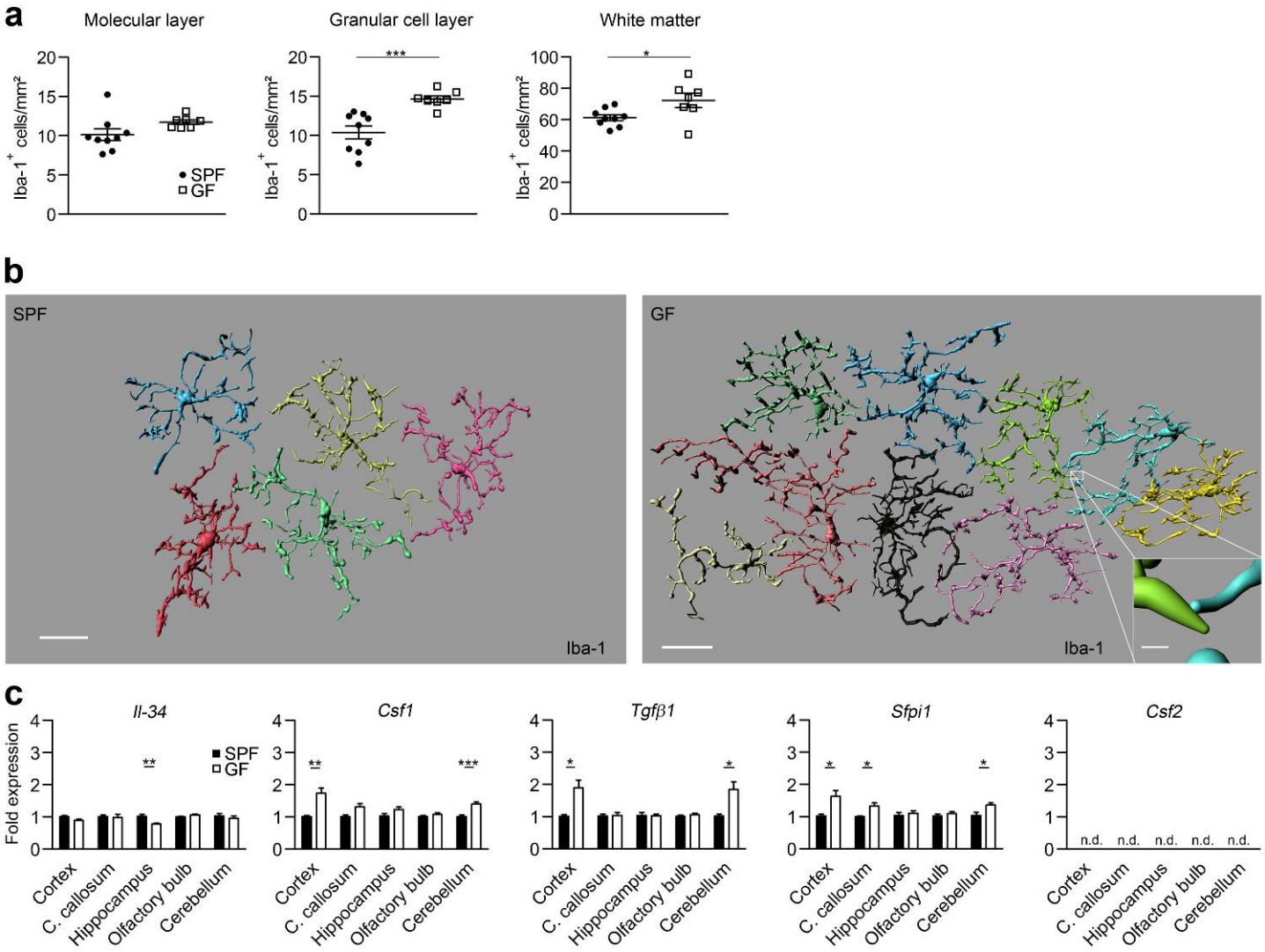
**(a,b)** Quantitative assessment of the neuroectodermal compartment of 6-8 weeks old GF or control SPF mice. Hippocampal $NeuN^+$ cells were counted in the gyrus dentatus. Cerebellar $NeuN^+$ cells were counted in the granular cell layer. Each symbol represents one mouse, with five samples per group. Means ± s.e.m. are indicated. No significant differences could be determined by using an unpaired *t* test. Representative pictures are shown. n.d. = not detectable. Data are representative of two independent experiments. Scale bars: H&E: 200 µm.




**Supplementary Figure 4**

Lymphocytic cells are indistinguishable in the brains of SPF and GF mice.

Immunohistochemistry for T cells (CD3) and B cells (B220) in different brain regions of SPF and GF mice, respectively. Insets depict spleen sections as positive controls. Scale bars: overviews: cortex, corpus callosum and cerebellum (CD3): 50 µm; olfactory bulb (CD3): 20 µm; hippocampus (CD3), cortex, corpus callosum, hippocampus, olfactory bulb and cerebellum (B220): 100 µm. Inset: 50 µm (CD3) and 100 µm (B220).



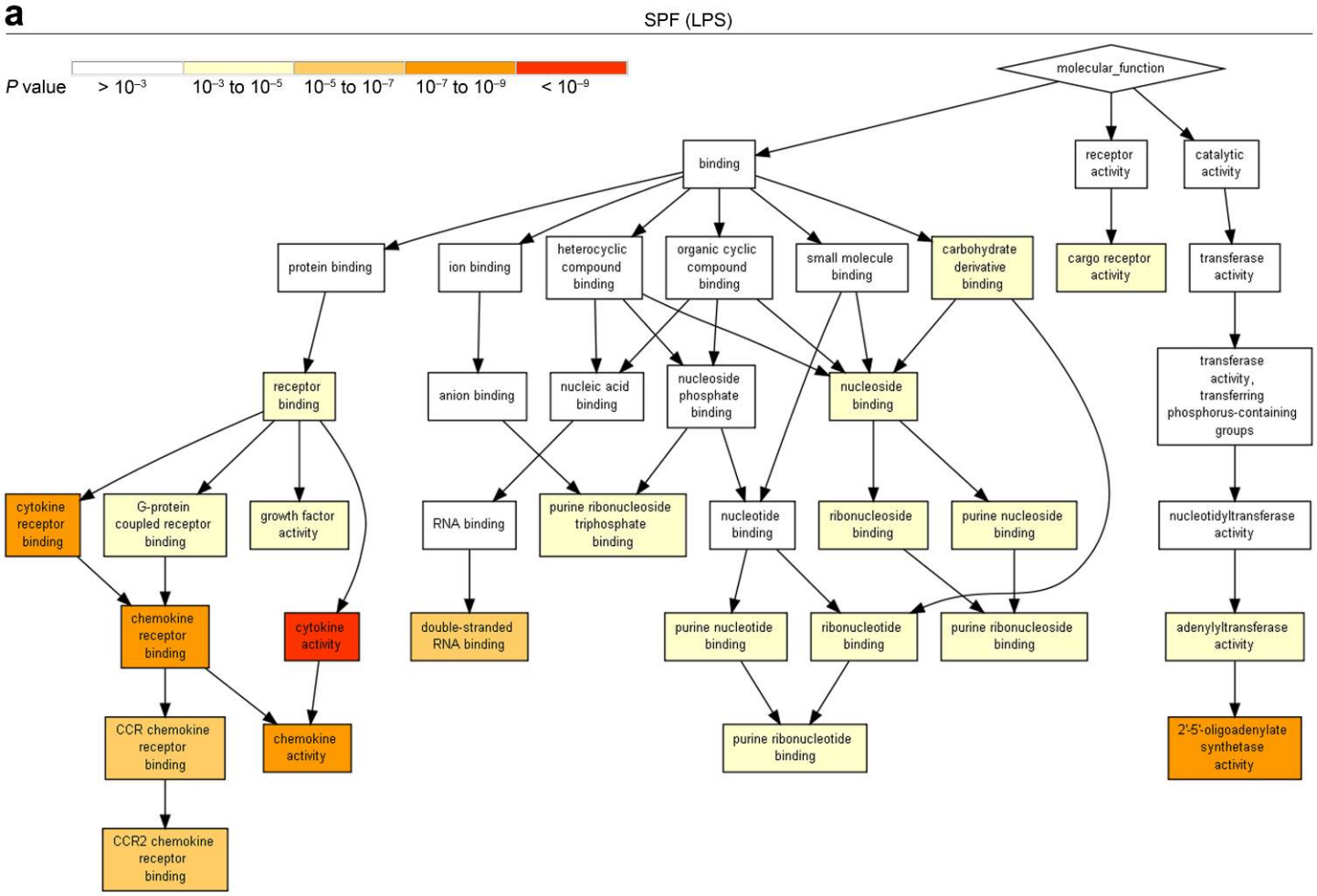

**Supplementary Figure 5**

Increased microglia number in cerebellar grey and white matter of GF mice.

**(a)** Quantification of Iba-1$^+$ parenchymal microglia from either SPF (black symbols) or GF (white symbols) mice was performed on cerebellar brain slices. Each symbol represents one mouse. Three to four sections per mouse were examined. Means ± s.e.m. are shown. Significant differences were determined by an unpaired *t* test and marked with asterisks (*$P$ < 0.05, ***$P$ < 0.001). *P* values: granular cell layer: 0.0008, white matter: 0.0287. Representative pictures of nine examined mice per group are displayed.

**(b)** Imaris-based 3-D reconstruction of the cortical microglia networks under SPF (left) or GF (right) conditions. GF microglia have no distances to neighbouring microglia and show partial physical contacts to adjacent cells. Inset exhibits such an interaction. Scale bars: 20 µm (overview), 3 µm (inset).

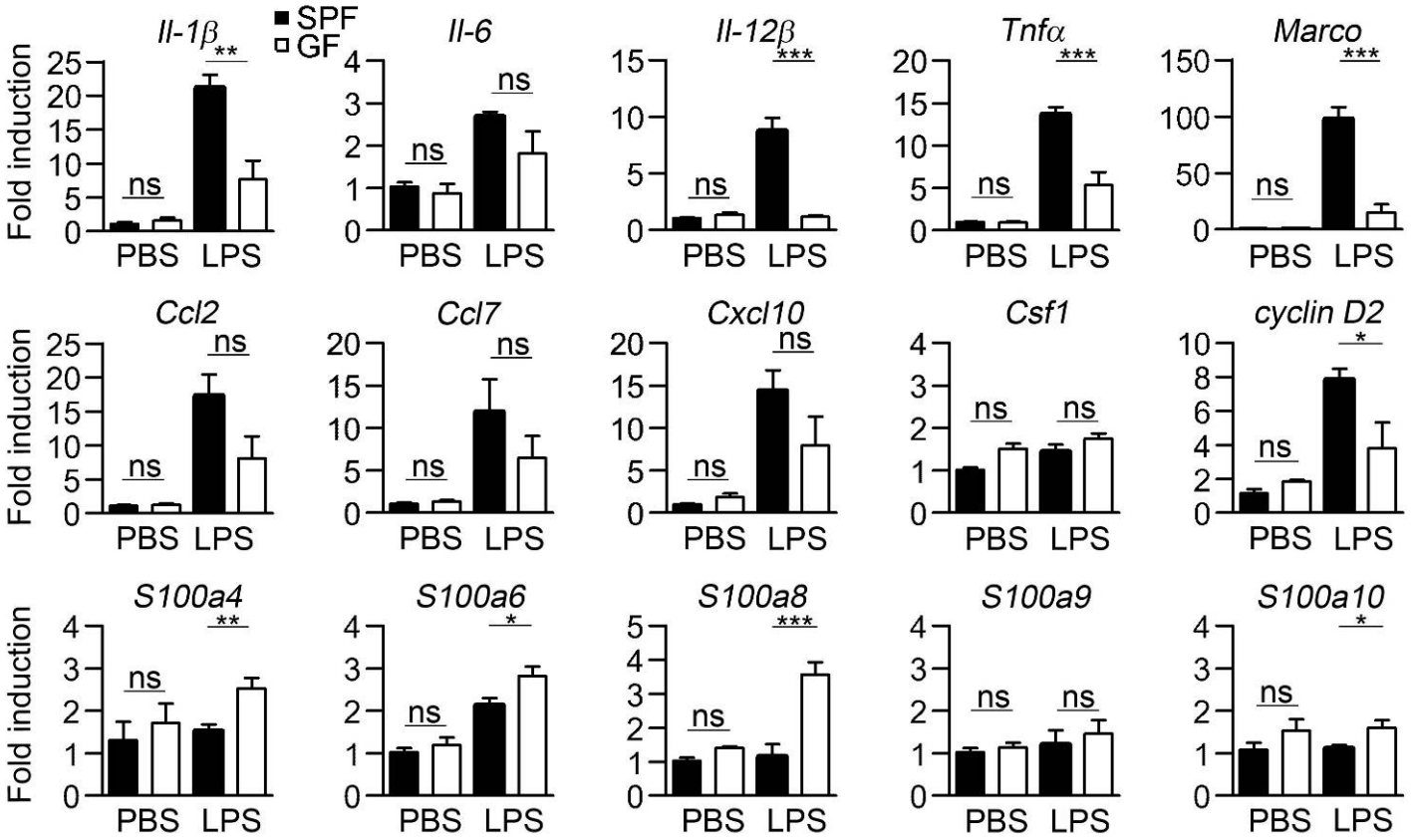
**(c)** Quantitative RT-PCR for the indicated mRNAs. Data are expressed as ratio of mRNA expression versus endogenous *Actb* relative to SPF conditions and exhibited as mean ± s.e.m. Bars (black: SPF, white: GF) represent means ± s.e.m. with at least four samples in each group. Data are representative of two independent experiments. Significant differences were determined by an unpaired *t* test and marked with asterisks (*$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001). n.d. = not detectable. *P* values: *Il-34*: hippocampus: 0.0031. *Csf1*: cortex: 0.0071, cerebellum: 0.0009. *Tgfß1*: cortex: 0.0253, cerebellum: 0.031. *Sfpi1*: cortex: 0.0302, corpus callosum: 0.0290, cerebellum: 0.0345.


a
SPF (LPS)
P value
> 10−3
10−3 to 10−5
10−5 to 10−7
10−7 to 10−9
< 10−9
molecular_function
binding
receptor activity
catalytic activity
protein binding
ion binding
heterocyclic compound binding
organic cyclic compound binding
small molecule binding
carbohydrate derivative binding
cargo receptor activity
transferase activity
receptor binding
anion binding
nucleic acid binding
nucleoside phosphate binding
nucleoside binding
transferase activity, transferring phosphorus-containing groups
cytokine receptor binding
G-protein coupled receptor binding
growth factor activity
RNA binding
purine ribonucleoside triphosphate binding
nucleotide binding
ribonucleoside binding
purine nucleoside binding
nucleotidyltransferase activity
chemokine receptor binding
cytokine activity
double-stranded RNA binding
purine nucleotide binding
ribonucleotide binding
purine ribonucleoside binding
adenylyltransferase activity
CCR chemokine receptor binding
chemokine activity
purine ribonucleotide binding
2'-5'-oligoadenylate synthetase activity
CCR2 chemokine receptor binding
b
GF (LPS)
molecular_function
binding
catalytic activity
protein binding
ion binding
isomerase activity
S100 protein binding
calcium-dependent protein binding
receptor binding
cation binding
intramolecular oxidoreductase activity
RAGE receptor binding
metal ion binding
intramolecular oxidoreductase activity, transposing C=C bonds
calcium ion binding
isopentenyl-diphosphate delta-isomerase activity

**Supplementary Figure 6**

Decreased inflammatory repertoire of microglia from GF mice upon intracerebral LPS challenge.

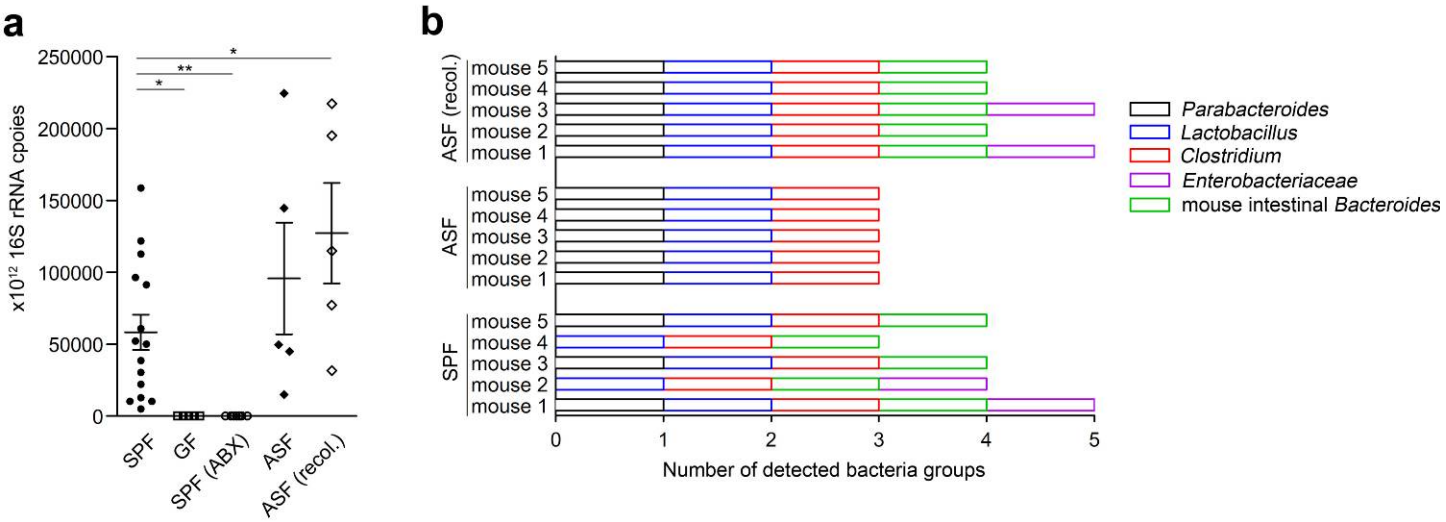
Gene ontology enrichment network on differentially expressed genes in microglia from SPF (**a**) or GF (b) mice on the basis of an Affymetrix DNA microarray analysis. Diagram depicts functional results of GO clustering through GORilla. Only very highly significantly overrepresented GO terms are included with *P* values ranging from $P < 10^{-9}$ (yellow) to $P < 10^{-24}$ (red). White framed terms depict not significantly overrepresented parent terms of the colored terms.




**Supplementary Figure 7**

Diminished inflammatory response of microglia from GF mice upon peripheral LPS challenge.

Quantitative RT-PCR of LPS-induced factors in microglia 6 hrs after intraperitoneal LPS challenge. Data are expressed as ratio of the mRNA expression compared to endogenous *Actb* relative to control and show mean ± s.e.m. At least three mice per group were analyzed. Data are representative of two independent experiments. Significant differences were determined by an unpaired *t* test and marked with asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). ns: not significant. *P* values: PBS: *Il1-β:* 0.3720, *Il-6*: 0.5625, *Il12β:* 0.2442, *Tnfα* p=0.5955, *Marco*: 0.0642, *Ccl2*: 0.6608, *Ccl7*: 0.3316, *Cxcl10*: 0.0867, *Csf1*: 0.1194, *cyclin D2*: 0.1135, *S100a4*: 0.5357, *S100a6*: 0.4281, *S100a8*: 0.0642, *S100a9*: 0.4583, *S100a10*: 0.1771. LPS: *Il-1β:* 0.0023, *Il-6*: 0.1329, *Il-12β:* <0.0001, *Tnfα*: 0.0008, *Marco*: 0.0001, *Ccl2*: 0.0673, *Ccl7*: 0.2708, *Cxcl10*:.1522, *Csf1*: 0.1828, *cyclin D2*: 0.0344, *S100a4*: 0.0064, *S100a6*: 0.0341, *S100a8*: 0.0009, *S100a9*: 0.5782, *S100a10*: 0.0352.

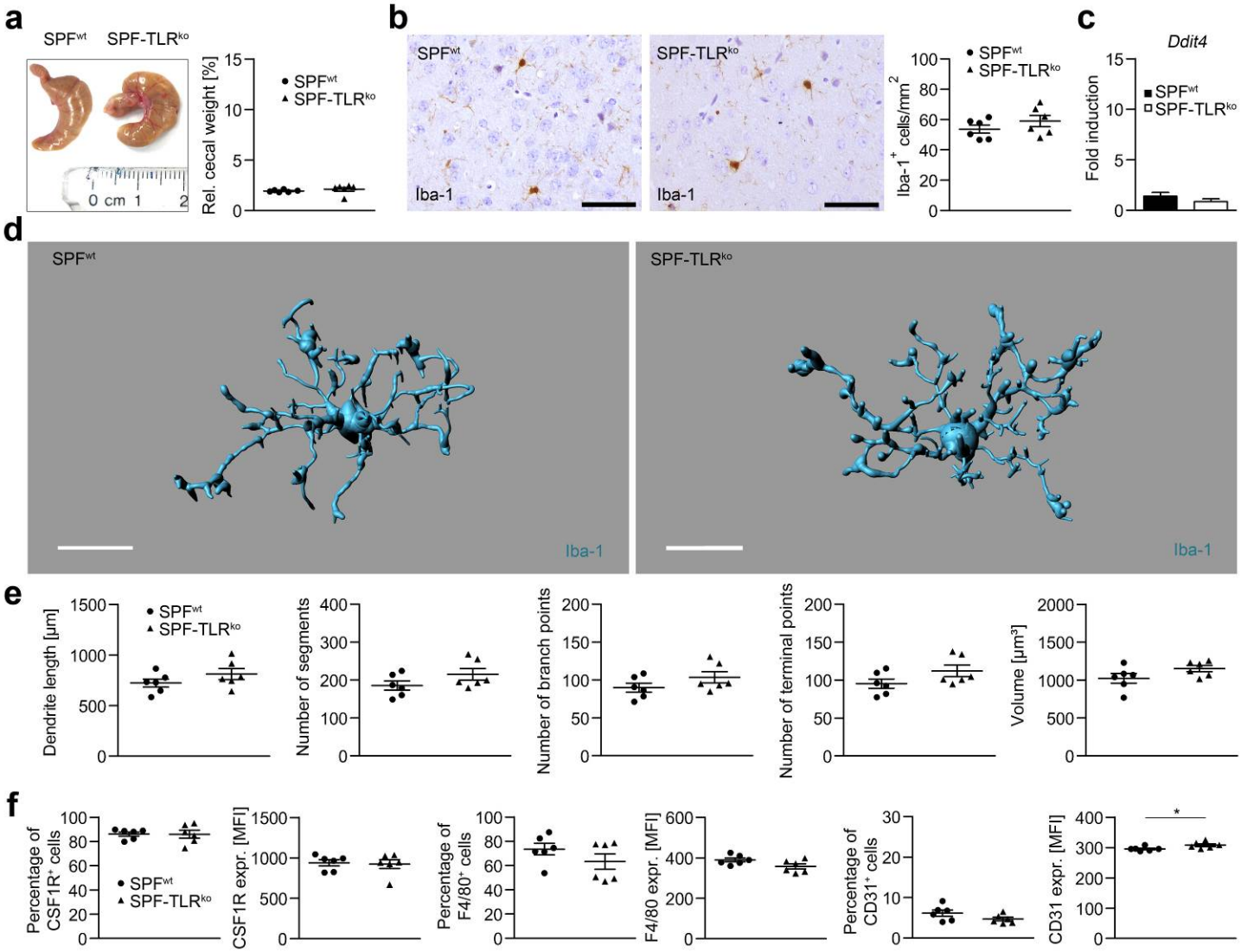



**Supplementary Figure 8**

Determination of intestinal bacterial loads and complexity.

**(a)** qPCR analysis for eubacterial 16S rRNA could not detect significant amounts of bacteria in the caeca of GF and antibiotic-treated SPF animals. Representative data from fifteen SPF, five GF, six SPF (ABX), five ASF and five ASF (recol.) mice are depicted. Gene copy numbers were normalized to DNA content. Each symbol represents one mouse. Means ± s.e.m. are indicated. Significant differences were determined by an unpaired *t* test and marked with asterisks (*$P < 0.05$, **$P < 0.01$). *P* values: SPF vs. GF: 0.0151, SPF vs. SPF (ABX): 0.0046, SPF vs. ASF (recol.): 0.0280.

**(b)** Recolonized ASF mice (ASF recol.) harbour a greater microbial diversity as detected by qPCR using group specific primers. Besides *Parabacteroides* (ASF357), *Lactobacillus* (ASF 361) and *Clostridium* (ASF356), mouse intenstinal *Bacteroides* (*mib)* and *Enterobacteriaceae* groups are detectable in the caecum of SPF and re-colonised ASF animals using group-specific primers, respectively.




**Supplementary Figure 9**

Microglia maturation is independent from the TLRs2,3,4,7,9.

**(a)** Left: View of caecum from a SPF-TLR$^{wt}$ (control, left) and SPF-TLR2,3,4,7,9-deficient (SPF-TLR$^{ko}$) mouse (right). Ruler scale is shown. Representative pictures are shown. Right: Relative caecum weight of control SPF-TLR$^{wt}$ and SPF-TLR$^{ko}$ mice. Symbols represent individual mice, with six animals per group. Means ± s.e.m. are shown. No statistically significant differences were found by an unpaired *t* test.

**(b)** Iba-1 immunohistochemistry in the cortex of SPF-TLR$^{wt}$ and SPF-TLR$^{ko}$ animals (left) and quantification thereof (right). Every symbol represents one mouse. Six animals per group were analyzed and three to four sections per mouse were examined. Data are representative of two independent experiments. Means ± s.e.m. are indicated. No significant differences could be determined by an unpaired *t* test.

**(c)** *Ddit4* mRNA measured by qRT-PCR in microglia from SPF-TLR$^{wt}$ (black bar) or SPF-TLR$^{ko}$ (white bar) mice. Means ± s.e.m. with six samples in each group are displayed. No significant differences could be determined by an unpaired *t* test. Data are representative of two independent experiments.

**(d)** Three-dimensional reconstruction (Scale bars: 15µm) and Imaris-based automatic quantification of cell morphometry (**e**) of Iba-1$^{+}$ microglia. Individual symbols represent one mouse per group with at least three measured cells per mouse. Six mice per group were analyzed. Means ± s.e.m. are indicated. No significant differences were detected by an unpaired *t* test.

**(f)** Flow cytometry of isolated microglia from control six SPF-TLR$^{wt}$ and six SPF-TLR$^{ko}$ individuals. Percentages and mean fluorescence

intensities (MFI) of the indicated surface molecules on microglia are shown. Each symbol represents one mouse. Means ± s.e.m. are indicated. Significant differences were evaluated by an unpaired *t* test and labelled with asterisks (*$P < 0.05$). *P* value: CD31 (MFI): 0.0419. Data are representative of two independent experiments.