

Supplementary

Supplementary methods

Ultrastructure (supplementary detailed protocol)

Immuno-electron microscopy tissue processing

Sections were washed in phosphate-buffered saline (PBS), quenched in 0.3% H₂O₂ (in PBS) for 10 minutes, washed, permeabilized in 0.1% NaBH₄ (in PBS) for 30 minutes, and washed again. After quenching and permeabilization, sections were placed for 1 hour at room temperature (RT) in blocking buffer (10% fetal bovine serum, 3% bovine serum albumin, 0.01% Triton X-100 in 50mM TBS, pH=7.6) and incubated overnight at 4°C with the primary antibody rabbit anti-Iba1 (1:1000 in BB; cat#019-19741, FUJIFILM Wako Chemical, Osaka, Japan). The next day, brains sections were washed in Tris-buffered saline (TBS), incubated for 1.5 hours at RT with the secondary antibody biotinylated goat anti-rabbit (1:300 in TBS; cat# 111-066-046, Jackson ImmunoResearch, West Grove, PA, United States), followed by the avidin-biotin complex (1:1:100 in TBS; cat# PK-6100 ,Vector Laboratories, Burlingame, CA, United States) for 1 hour at RT, then washed and revealed in 0.05% diaminobenzidine (DAB, 0.015% H₂O₂, in TBS; cat# D5905-50TAB, MilliporeSigma). Sections were next processed for electron microscopy (EM). Tissues were incubated in 3% ferrocyanide (in H₂O; cat# PFC232.250, BioShop, Burlington, ON, Canada) combined (1:1) with 4% aqueous osmium tetroxide (cat#19170, Electron Microscopy Sciences, Hatfield, PA, United States) for 1 hour, washed in PBS, incubated in 1% thiocarbohydrazide (in PBS; cat# 2231-57-4, Electron Microscopy Sciences) for 20 minutes, washed in PBS, incubated in 2% osmium tetroxide (in H₂O), then dehydrated in ascending concentration of ethanol (35%, 50%, 70%, 80%, in 90%, in 100%) followed by incubation in propylene oxide. Post-fixed sections were embedded in Durcupan ACM resin (cat# 44611-44614, MilliporeSigma) for 24 hours, placed between two ACLAR® embedding sheets (cat# 50425-25, Electron Microscopy Sciences) and resin was polymerised at 55°C for 72 hours. Region of interest –the barrel cortex– was excised, glued on a resin block, and cut into 75nm-thick ultrathin sections using a Leica Ultracut UC7 ultramicrotome (Leica Biosystems).

Ultrastructure characterisation

Dilation of the endoplasmic reticulum and Golgi apparatus cisternae was identified when the space between both cisternae membranes was greater than 50 nm¹. Lysosomes were distinguished by their heterogenous electron-dense round structure, which was subdivided into primary, secondary (when associated with endosomes) and tertiary (when associated with lipidic inclusions and often with endosomes) categories^{2,3}. Lipidic inclusions regrouped lipofuscins as well as lipidic bodies. Lipofuscins were recognised by their electron-dense spherical structure with a distinct fingerprint-like pattern, whereas lipid bodies showed a smooth and uniform pattern³. Mitochondrial elongation was identified by a length of 1 µm or greater¹. Microglia were recognized by their immunoreactivity to Iba1 as well as ultrastructural features including: their dark irregular nuclei with a heterogenous chromatin pattern and dark irregular cytoplasm, often containing long stretch of endoplasmic reticulum cisternae and lipidic inclusions (i.e., lipofuscin, lipid bodies, lipid droplets and lysosomes)⁴. Neurons were distinguished by their pale nuclei and pale cytoplasm, often with an apical dendrite and synaptic contacts⁴ (**Supplementary Figure 7f**). Synapses were identified by a visible synaptic density between the presynaptic axon terminal containing synaptic vesicles and the postsynaptic dendritic spine⁴ (**Supplementary Figure 1c**). Astrocytic cells were identified by their pale nuclei with a thin rim of heterochromatin and their pale irregular cytoplasm, often containing intermediate filaments⁴. Glycogen granules were recognised as dark electron-dense granules⁴ (**Supplementary Figure 7g**). Oligodendrocytes were distinguished by their dark round or oval nuclei with a heterogenous chromatin pattern and their dark squared-shape wide cytoplasm containing short, wide endoplasmic reticulum cisternae and often enriched in ribosomes⁴ (**Supplementary Figure 7h**). Capillaries were counted when microglial cell bodies directly touched their basement membrane (**Supplementary Figure 1c**), which forms a thin electron-dense layer encompassing the capillary's cells including endothelial cells, pericytes and other perivascular cells (**Supplementary Figure 7a**). Digestive exophagy, also known as extracellular digestion, was identified by extracellular space pockets containing degraded elements or debris in areas directly adjacent to the microglial cell body⁵⁻⁷ (**Supplementary Figure 7j**). Degraded myelin was recognized by ballooning, swelling or distancing between the well-defined myelin sheaths⁴ and was often observed with exophagy (**Supplementary 7j**).

Behaviours.

For each behavioural test, animals were acclimated to the experimental room for 30 minutes prior to testing under the experimental lighting condition. All behaviours were assessed in a way to prevent mice from seeing the experimenter during testing. Between each trial and tests, arena and objects were thoroughly cleaned with ethanol/peroxide mixture to prevent any olfactory cues. Except for the open field, marble burying and prepulse inhibition (PPI), all behavioural tests were filmed and scored automatically by the tracking software TopScan Version 2.00 (Clever Sys Inc, Reston, VA, United States), and scoring was verified by an observer blinded to experimental conditions.

Open field

Mice were placed in a transparent Plexiglas open field (40cm (width) x 40cm (length) x 30cm (height)) connected to VersaMax system (*Accuscan Instruments Inc.*, Columbus, OH, United States) for 15 minutes after the dark phase (between 8:00 and 11:00). The test was divided into first 5 minutes of habituation and 10 minutes testing. VersaMax tracking system automatically recorded global locomotion parameters: speed, distance travelled, time spent in different areas of the arena (i.e., centre *versus* edge *versus* corner), horizontal and vertical movements, clockwise and anti-clockwise rotations, as well as stereotypic behaviours.

Novel Object Recognition

Novel object recognition consisted of a training and a testing phase in an opaque plexiglass box (40cm (width) x 40cm (length) x 30cm (height)) under the infrared light in the beginning of light phase (between 8:00 and 11:00). Mice were, first, let to explore two identical objects for 5 minutes during the acquisition phase. Then, the animals were put back in their home cage for 30 minutes. During the novel object recognition retention phase, mice were placed in the same experimental arena for 5 minutes in the presence of one of the two familiar objects along with a novel object of similar size. The novel object was randomly assigned. Object exploration was defined as touching or sniffing within the interaction zone (within ~2cm of the object). The recognition index was calculated by the ratio of the time spent exploring the object over

the total time allowed. Mice with an exploration time lower than 10 seconds per object were considered unsuitable and were not used.

Three-chambers social interaction

For two days prior to testing, stranger mice were habituated to the wired cage under infrared light for 20 minutes to minimise animal stress during social interaction assessment. Experimental mice were placed for 10 minutes in the central compartment and let free to explore the 3-chambers compartment (chamber: 26 cm (length) 21.6 (width) x 21.6 (height); door: 5 cm (width) x 5 cm (height)) with the two wired cages in the two side compartments. After the habituation to the three-chambers, a stranger mouse was placed in a cylindrical wired-cup (dimension: 7.6 cm (D) x 9.5 cm (H)) in one of the two side compartments and a toy in the other one. Social preference was assessed for 10 minutes, then the toy was substituted with a novel stranger to evaluate social novelty preference for another 10 minutes session. Time spent and number of entries in each compartment, as well as contacts/sniffing time with the occupied wired cage were measured using TopScan software. Social preference index and social novelty index were respectively calculated by the ratio of time interacting with stranger over toy, and time interacting with novel over familiar stranger.

Elevated plus maze

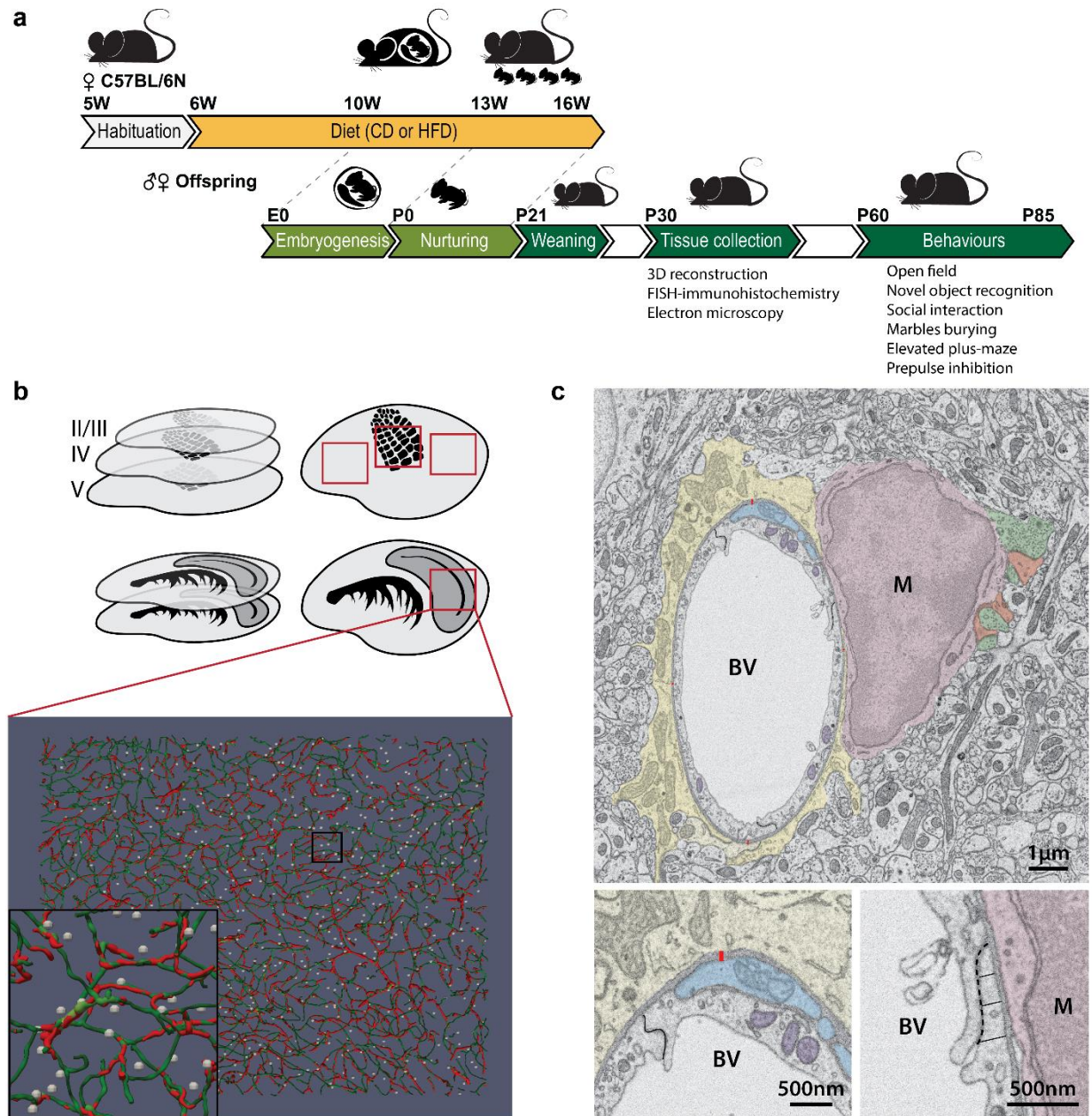
Elevated plus maze was assessed at the beginning of the light phase (8:00-11:00) under normal lighting (~30 lux). Mice were placed at the junction of the open and closed arms facing the open arms and let free to explore the elevated plus maze (open arm: 29.25 cm (length) x 5 cm (width); closed arm: 29.25 cm (length) x 5 cm (width) x 11.5 cm (height); centre: 5 cm (width) x 5 cm (length)) for 10 minutes. After the 5 minutes session, animal was put back in its home cage. Time spent and number of entries in open arms, closed arms and at the centre were measured to evaluate anxiety-related behaviour at basal level using TopScan software. Entry was considered when all three paws entered the arm.

Prepulse inhibition (PPI)

PPI is the inhibition of the startle response to a strong auditory stimulus when the stimulus is preceded by a weaker stimulus⁸. Alterations of the PPI are known to be commonly observed in neurodevelopmental disorders, we measured the sensorimotor gating which is altered in neurodevelopmental disorders, such as

schizophrenia⁸. Animals were placed in a cylindrical Plexiglas animal enclosure inside a commercially available system (SR-LAB; San Diego Instruments, San Diego, CA, United States). After 5 minutes of acclimation inside the enclosure, 42 discrete trials were performed; the first two trials are 120dB in magnitude followed by 40 trials in which the startle pulse was either alone or 100ms prior to a 30ms prepulse. The prepulses of intensity ranging from 3 to 15 dB were randomly presented to experimental mice. Startle responses were determined automatically by the SR-LAB system. PPI was calculated as the relative percentage of the mean amplitude of the startle response without prepulse compared to those recorded following a prepulse.

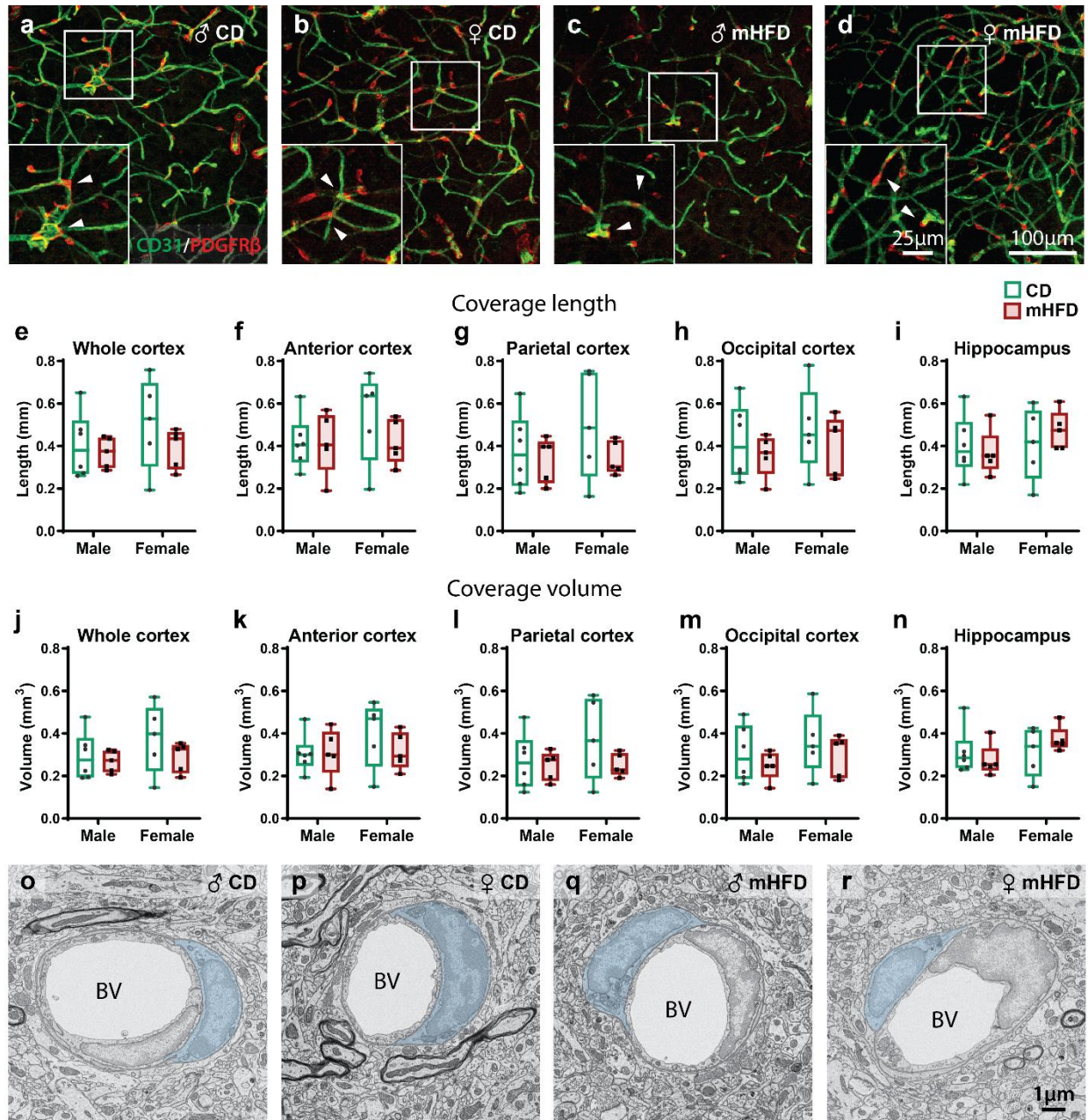
Supplementary figures



Supplementary Figure 1. Schematic of mHFD experimental design and neurovascular unit analysis.

(a) Schematic illustrates diet protocol on the C57BL/6N dams (starting at 5 weeks old up to 16 weeks old, at weaning of their litter), as well as experimental protocol on offspring (mHFD exposure from E0 to P21, tissue collection at P30, and behavioural assessment from P60 to P85). (b) At P30, tissues were notably collected for analysis of the vascular unit organisation and structure via (b) 3D-reconstruction and (c) electron microscopy. (b) Flatten-brains were imaged on three sections of cortices (layer II/III, layer IV, layer

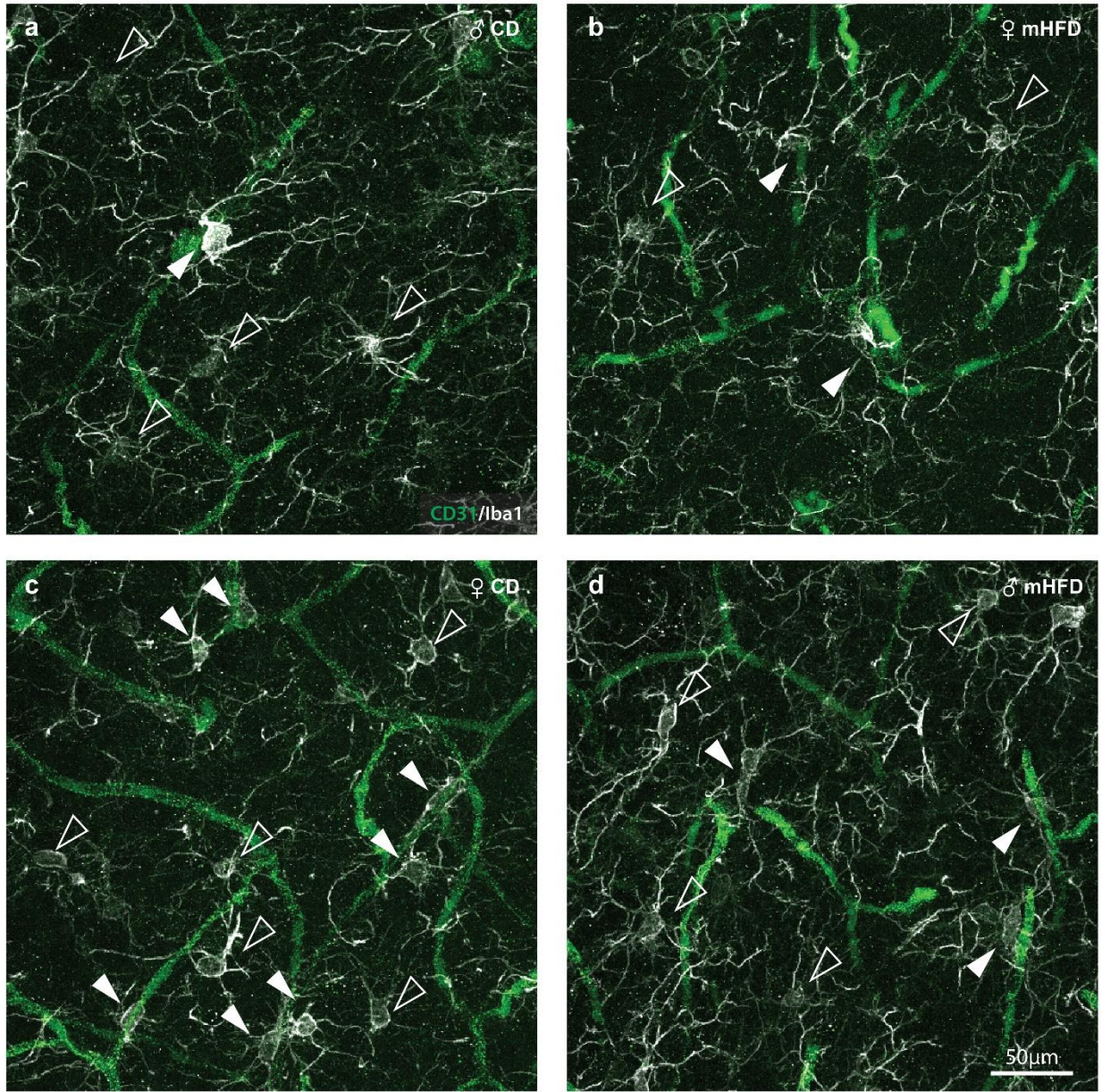
V of the anterior, parietal, and occipital cortices) and two sections of the CA1 hippocampus for 3D-reconstruction. The example of 3D-reconstruction shows endothelial cells in green, pericytes in red, and microglial cell bodies in white. (c) At nanoscale level, the ultrastructure of capillaries (identified by “BV”) and microglia (identified by “M”, pseudocoloured in fuchsia) were characterized. Number of astrocytic endfeet (pseudocoloured in yellow), pericytic processes (in light blue), tight junctions (highlighted by a fine black line) and mitochondria (in purple) were quantified. Tight junction length (dotted line) and thickness (fine grey lines) as well as basement membrane thickness (thick red lines) were measured. Similarly, microglial organelles and interactions with their microenvironment were analysed; here, we can see the microglia making a direct contact with the basement membrane of the capillary as well as several contacts with presynaptic axon terminals (pseudocoloured in green) and postsynaptic dendritic spines (pseudocoloured in red). ♀: female, ♂: male, CD: control diet, E: embryonic day, HFD: maternal high-fat diet, P: postnatal day, W: week.



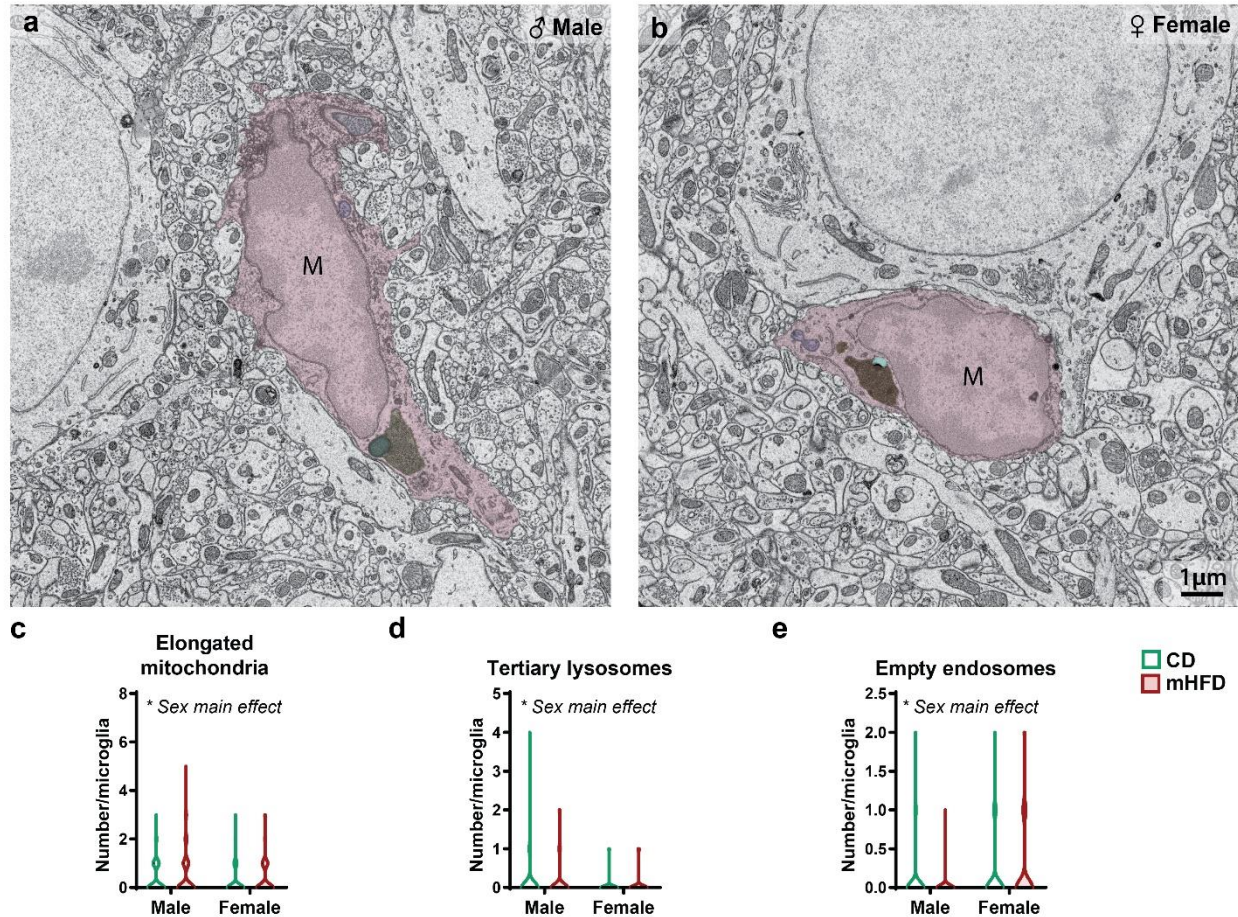
Supplementary Figure 2. mHFD had no significant effect on cortical and hippocampal pericyte coverage in P30 male and female offspring. (a-d) Pictures at 20x with an epifluorescence microscope illustrate pericyte coverage of the blood vessels from the parietal cortex of adolescent offspring, where white arrows indicate colocalisation on the inset. (e-i) Pericyte coverage length and (j-n) coverage volume were measured for the (e, j) whole cortex, (f, k) anterior cortex, (g, l) parietal cortex, (h, m) occipital cortex and (i, n) hippocampus of the adolescent offspring (n=5-6 animals/diet/sex). (o-r) At the ultrastructural level,

pericytes (pseudocoloured in light blue) are well anchored to the capillary (identified “BV”) (n=93-95 capillaries/diet/sex, N=4 animals). Whisker graphs show minimum, median (line) and maximum, as well as individual data points. ♀: female, ♂: male, CD: control diet, mHFD: maternal high-fat diet.

at 20x with an epifluorescence microscope illustrate Iba1⁺ microglia (in white) cell density and their distribution from the (a-d) anterior cortex and (e-h) parietal cortex of adolescent offspring. (i-m) Density, (n-r) nearest neighbour distance and (s-w) spacing index were determined for the (i, n, s) whole cortex, (j, o, t) anterior cortex, (k, p, u) parietal cortex, (l, q, v) occipital cortex and (m, r, w) hippocampus of the adolescent offspring. Whisker graphs show minimum, median (black line) and maximum, as well as individual data points (n=5-6 animals/diet/sex). * $P < 0.05$ by mixed-effect analysis identifying a sex effect. ♀: female, ♂: male, a.u.: arbitrary unit, CD: control diet, mHFD: maternal high-fat diet NND: nearest neighbour distance.

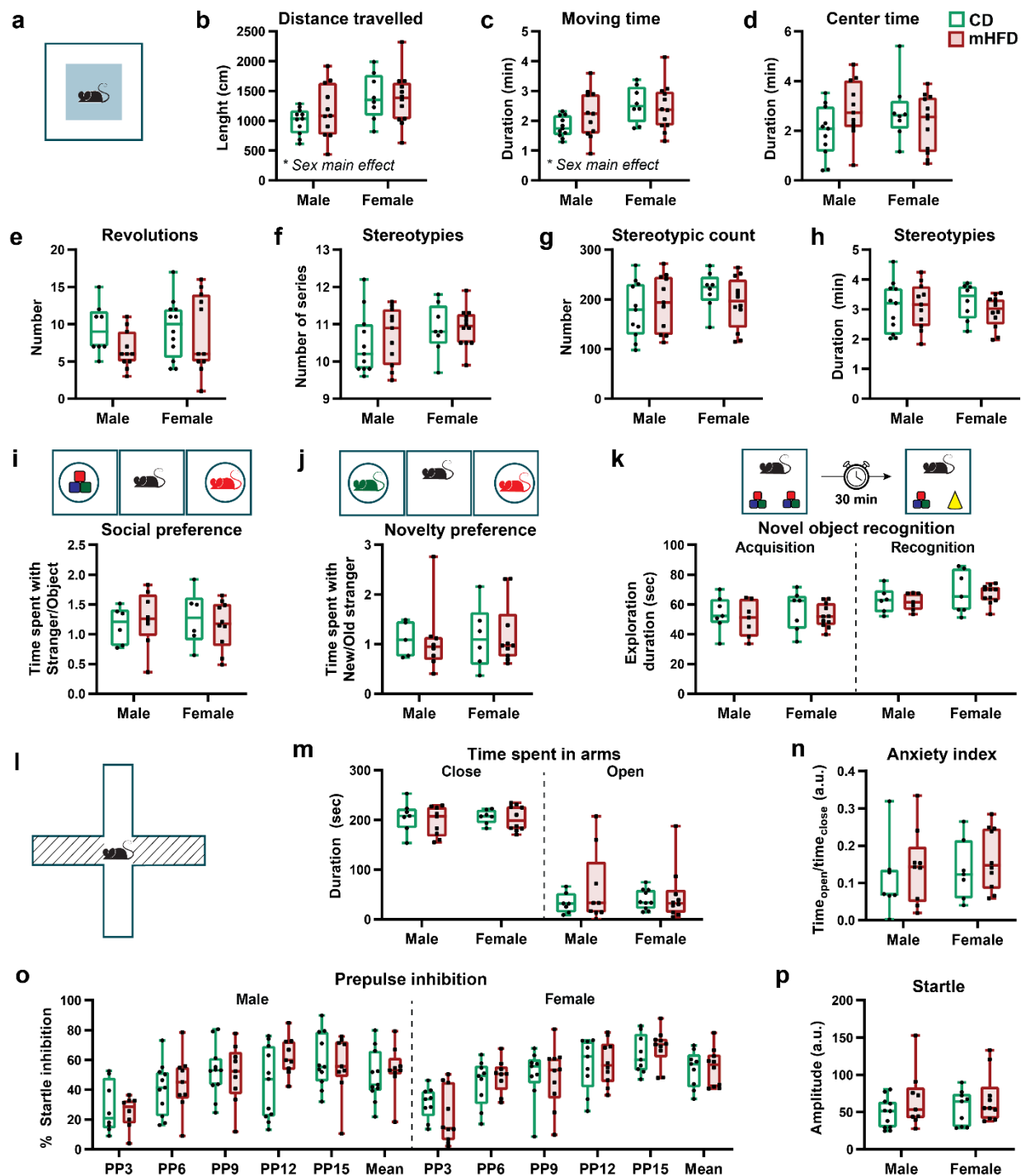


Supplementary Figure 4. Effects of mHFD on microglial distance to blood vessels and their putative contacts with blood vessels in CA1 hippocampus of P30 offspring. (a-d) Pictures taken at 63x by a confocal microscope show microglia interaction with blood vessels from the CA1 hippocampus, where microglia making putative contacts (filled white arrowheads) and microglia that are not making putative contacts (open white arrowheads) are identified. ♀: female, ♂: male, CD: control diet, mHFD: maternal high-fat diet.



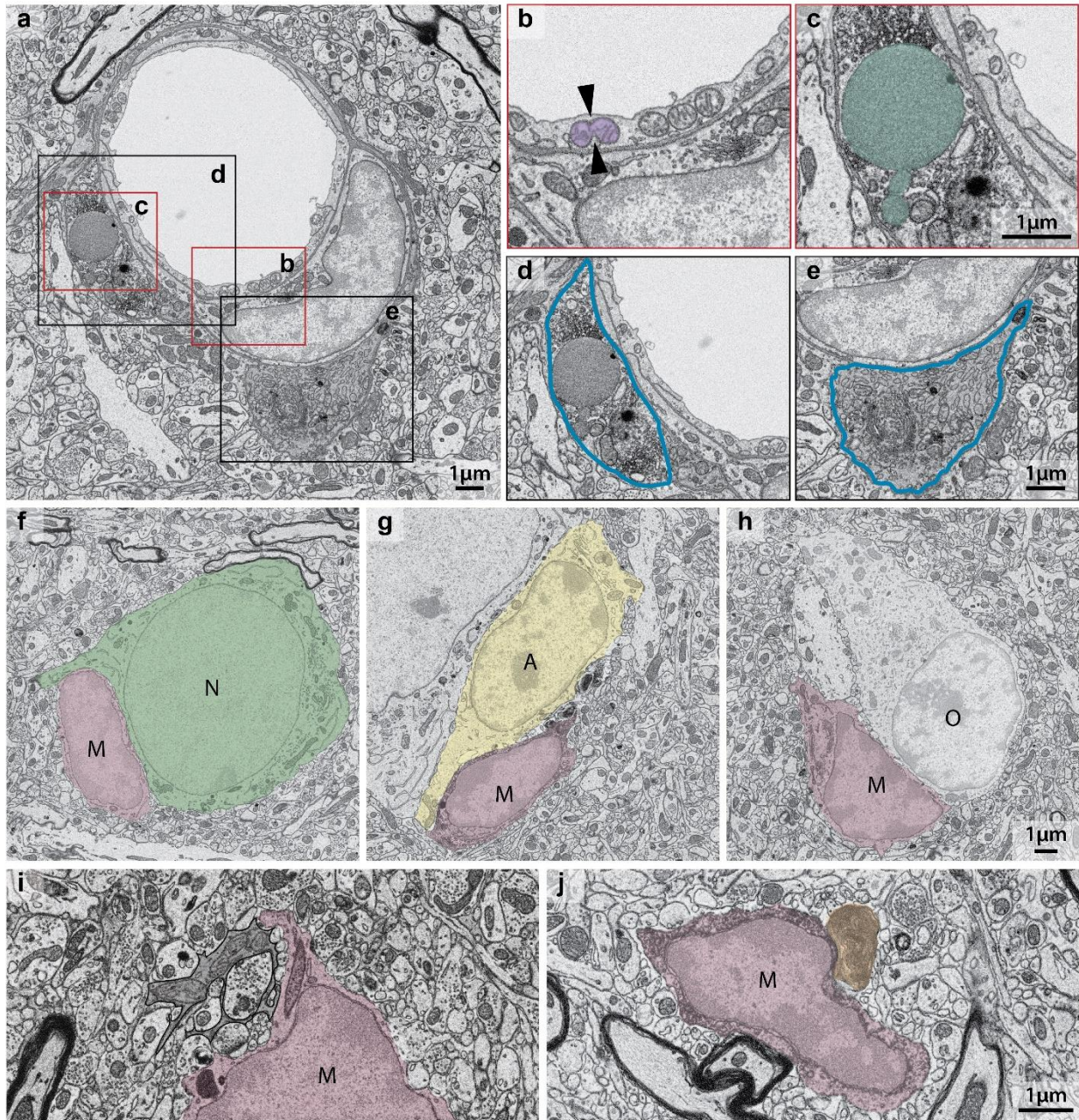
Supplementary Figure 5. Microglia from the parietal cortex exhibit ultrastructural sex differences.

(a-b) Micrographs illustrate main ultrastructural features of microglia (identified by “M”, pseudocoloured in fuchsia) presenting sex differences regardless of their maternal diet, in which mitochondria are pseudocoloured in purple, elongated mitochondria in light purple, tertiary lysosomes in orange, primary and secondary lysosomes in dark orange, lipid body in turquoise and empty endosome in teal. Notably, microglia from male offspring cortex had an increased number of (c) elongated mitochondria, (d) tertiary lysosomes and (e) decreased number of empty endosomes. Violin graphs show minimum, median (black line) and maximum. $^{\#}P < 0.05$ by mixed-effect analysis identifying a sex effect. ♀: female, ♂: male, CD: control diet, mHFD: maternal high-fat diet.



Supplementary Figure 6. The effect of mHFD on motor function, spatial memory, social interaction, anxiety-like behaviours, and sensorimotor gating in adult male and female adult offspring. Schemes of the experimental paradigm of (a) open field, (i) elevated plus-maze, (l-m) three-chambers test and (n) novel object recognition test are illustrated. (b) Distance travelled, (c) moving time, (d) time spent in the

centre, (e) number of revolutions, (f) stereotypies, (g) stereotypic count and (h) stereotypies number were measured by open field to assess global motor function. (k) Anxiety index was calculated with (j) time spent in arms during elevated plus-maze test. (l) Social preference and (m) novelty preference were evaluated using three-chambers test. (n) Spatial working memory was appraised by novel object recognition. (o) Sensorimotor gating of the acoustic startle response and (p) acoustic startle response were measured by the prepulse inhibition test. Whisker graph shows minimum, median (line) and maximum, as well as individual data points (n=6-10 litters/diet/sex). * $P < 0.05$ by 2-way ANOVA analysis : female, ♂: male, a.u.: arbitrary unit, CD: control diet, mHFD: maternal high-fat diet.



Supplementary Figure 7. Examples of other ultrastructural parameters analysed. (a) A lower magnification of a capillary in which we can observe (b) fusion or fission of mitochondria pseudocoloured in purple, (c) lipid bodies pseudocoloured in turquoise as well as (d) Iba1+ cells (edge of the cell trace in blue) and (e) other undistinguished perivascular cells (edge of the cell trace in blue) within the perivascular space of capillaries. (f-i) Following panels illustrate microglial interactions with other cells of the brains, including (f) neuron (identified “N”, pseudocoloured in green), (g) astrocyte (identified “A”, pseudocoloured in yellow), (h) neuron (identified “N”, pseudocoloured in green), (i) neuron (identified “N”, pseudocoloured in green), and (j) neuron (identified “N”, pseudocoloured in green). Each panel includes a 1 μm scale bar.

pseudocoloured in yellow) and (h) oligodendrocyte (identified “O”, pseudocoloured in white), as well as (i) darker cell processes (edge of the process are highlighted by a black line), and (j) degraded myelin (pseudocoloured in orange). For each panel, microglia are pseudocoloured in fuchsia and identified by a “M” when visible.

Supplementary tables

Supplementary Table 1. Summary of ANOVAs test to assess *Diet* and *Sex* main effects as well as *Diet*Sex* interaction effect of 2D and 3D analyses of the neurovascular unit data. Data are presented as Mean $\pm$ SEM for each treatment group. *F* and *P* values are given for main and interactions effects (n=5-6 animals/diet/sex, 2-3 sections). ♂: male, ♀: female, a.u.: arbitrary unit, CD: control diet, mHFD: maternal high-fat diet, SEM: standard error of the mean.

Parameters			Mean $\pm$ SEM	<i>F</i>	<i>P</i>
Whole cortex	Blood vessels	Vessel length (mm/mm ³)	♂-CD: 1.672 $\pm$ 0.048 ♂-mHFD: 1.997 $\pm$ 0.047 ♀-CD: 1.647 $\pm$ 0.088 ♀-mHFD: 1.798 $\pm$ 0.067	<i>Diet*Sex</i> : 1.900 <i>Sex</i> : 3.095 <i>Diet</i> : 14.06	<i>Diet*Sex</i> : 0.1860 <i>Sex</i> : 0.0965 <i>Diet</i> : 0.0016 **
		Number of branching points (/mm ³)	♂-CD: 2.950 $\pm$ 0.161 ♂-mHFD: 4.040 $\pm$ 0.179 ♀-CD: 3.393 $\pm$ 0.217 ♀-mHFD: 4.033 $\pm$ 0.265	<i>Diet*Sex</i> : 1.193 <i>Sex</i> : 1.122 <i>Diet</i> : 17.63	<i>Diet*Sex</i> : 0.2900 <i>Sex</i> : 0.3044 <i>Diet</i> : 0.0006***
		Tortuosity index (a.u.)	♂-CD: 1.791 $\pm$ 0.027 ♂-mHFD: 1.832 $\pm$ 0.027 ♀-CD: 1.784 $\pm$ 0.011 ♀-mHFD: 1.799 $\pm$ 0.018	<i>Diet*Sex</i> : 0.3285 <i>Sex</i> : 0.7602 <i>Diet</i> : 1.550	<i>Diet*Sex</i> : 0.5740 <i>Sex</i> : 0.3954 <i>Diet</i> : 0.2301
	Pericytes	Length coverage (mm)	♂-CD: 0.404 $\pm$ 0.063 ♂-mHFD: 0.370 $\pm$ 0.033 ♀-CD: 0.506 $\pm$ 0.097 ♀-mHFD: 0.390 $\pm$ 0.042	<i>Diet*Sex</i> : 0.4108 <i>Sex</i> : 0.9169 <i>Diet</i> : 1.381	<i>Diet*Sex</i> : 0.5301 <i>Sex</i> : 0.3517 <i>Diet</i> : 0.2562
		Volume coverage	♂-CD: 0.293 $\pm$ 0.046 ♂-mHFD: 0.269 $\pm$ 0.023 ♀-CD: 0.377 $\pm$ 0.073 ♀-mHFD: 0.289 $\pm$ 0.032	<i>Diet*Sex</i> : 0.4603 <i>Sex</i> : 1.189 <i>Diet</i> : 1.393	<i>Diet*Sex</i> : 0.5066 <i>Sex</i> : 0.2907 <i>Diet</i> : 0.2542
	Microglia	Microglia density ratio (a.u.)	♂-CD: 0.929 $\pm$ 0.132 ♂-mHFD: 1.052 $\pm$ 0.044 ♀-CD: 1.085 $\pm$ 0.061 ♀-mHFD: 1.040 $\pm$ 0.108	<i>Diet*Sex</i> : 0.7117 <i>Sex</i> : 0.5263 <i>Diet</i> : 0.1563	<i>Diet*Sex</i> : 0.4106 <i>Sex</i> : 0.4780 <i>Diet</i> : 0.6975
		Microglial nearest distance to blood vessel (μ m)	♂-CD: 6.676 $\pm$ 0.253 ♂-mHFD: 5.853 $\pm$ 0.114 ♀-CD: 5.764 $\pm$ 0.216 ♀-mHFD: 5.282 $\pm$ 0.086	<i>Diet*Sex</i> : 0.7873 <i>Sex</i> : 14.94 <i>Diet</i> : 11.57	<i>Diet*Sex</i> : 0.3873 <i>Sex</i> : 0.0012** <i>Diet</i> : 0.0034**
		Ratio microglia in contact with blood vessel (a.u.)	♂-CD: 0.473 $\pm$ 0.016 ♂-mHFD: 0.520 $\pm$ 0.007 ♀-CD: 0.529 $\pm$ 0.017	<i>Diet*Sex</i> : 0.1134 <i>Sex</i> : 15.88 <i>Diet</i> : 10.57	<i>Diet*Sex</i> : 0.7405 <i>Sex</i> : 0.0010*** <i>Diet</i> : 0.0047**

Anterior cortex			♀-mHFD: 0.567 ± 0.008		
		Microglial density (cell/mm ²)	♂-CD: 6.542 ± 0.513 ♂-mHFD: 6.682 ± 0.260 ♀-CD: 6.829 ± 0.205 ♀-mHFD: 5.972 ± 0.628	Diet*Sex: 1.228 Sex: 0.2204 Diet: 0.6360	Diet*Sex: 0.2833 Sex: 0.6447 Diet: 0.4361
		NND (mm)	♂-CD: 24.86 ± 1.13 ♂-mHFD: 23.88 ± 0.41 ♀-CD: 23.66 ± 0.37 ♀-mHFD: 25.29 ± 1.13	Diet*Sex: 2.155 Sex: 0.01373 Diet: 0.1312	Diet*Sex: 0.1603 Sex: 0.9081 Diet: 0.7217
		Spacing index (a.u.)	♂-CD: 0.379 ± 0.005 ♂-mHFD: 0.370 ± 0.004 ♀-CD: 0.374 ± 0.004 ♀-mHFD: 0.363 ± 0.009	Diet*Sex: 0.03944 Sex: 1.095 Diet: 2.705	Diet*Sex: 0.8449 Sex: 0.3100 Diet: 0.1184
	Blood vessels	Vessel length (mm/mm ³)	♂-CD: 1.689 ± 0.072 ♂-mHFD: 2.017 ± 0.065 ♀-CD: 1.697 ± 0.142 ♀-mHFD: 1.849 ± 0.109	Diet*Sex: 0.7908 Sex: 0.6465 Diet: 5.804	Diet*Sex: 0.3863 Sex: 0.4325 Diet: 0.0276*
		Number of branching points (/mm ³)	♂-CD: 3.155 ± 0.254 ♂-mHFD: 4.425 ± 0.332 ♀-CD: 3.661 ± 0.416 ♀-mHFD: 4.229 ± 0.387	Diet*Sex: 1.032 Sex: 0.2015 Diet: 7.083	Diet*Sex: 0.3238 Sex: 0.6592 Diet: 0.0164*
		Tortuosity index (a.u.)	♂-CD: 1.810 ± 0.039 ♂-mHFD: 1.839 ± 0.046 ♀-CD: 1.791 ± 0.047 ♀-mHFD: 1.823 ± 0.026	Diet*Sex: 0.001931 Sex: 0.6659 Diet: 0.4547	Diet*Sex: 0.9655 Sex: 0.6659 Diet: 0.4547
	Pericytes	Length coverage (mm)	♂-CD: 0.418 ± 0.050 ♂-mHFD: 0.415 ± 0.066 ♀-CD: 0.539 ± 0.096 ♀-mHFD: 0.419 ± 0.047	Diet*Sex: 0.7560 Sex: 0.8830 Diet: 0.8549	Diet*Sex: 0.3967 Sex: 0.3605 Diet: 0.3681
		Volume coverage	♂-CD: 0.306 ± 0.037 ♂-mHFD: 0.310 ± 0.051 ♀-CD: 0.399 ± 0.071 ♀-mHFD: 0.318 ± 0.039	Diet*Sex: 0.7225 Sex: 1.020 Diet: 0.5795	Diet*Sex: 0.4071 Sex: 0.3267 Diet: 0.4569
	Microglia	Microglia density ratio (a.u.)	♂-CD: 0.954 ± 1.55 ♂-mHFD: 1.053 ± 0.063 ♀-CD: 1.055 ± 0.112 ♀-mHFD: 0.920 ± 0.139	Diet*Sex: 0.8436 Sex: 0.01526 Diet: 0.02011	Diet*Sex: 0.3712 Sex: 0.9031 Diet: 0.8889
		Microglial nearest distance to blood vessel (μm)	♂-CD: 6.672 ± 0.275 ♂-mHFD: 5.487 ± 0.180 ♀-CD: 5.647 ± 0.159 ♀-mHFD: 5.247 ± 0.111	Diet*Sex: 3.720 Sex: 9.655 Diet: 15.16	Diet*Sex: 0.0706 Sex: 0.0064 ** Diet: 0.0012**
			♂-CD: 0.477 ± 0.016 ♂-mHFD: 0.549 ± 0.013	Diet*Sex: 2.490 Sex: 9.325	Diet*Sex: 0.1330 Sex: 0.0072**

Parietal cortex		Ratio microglia in contact with blood vessel (a.u.)	♀-CD: 0.540 ± 0.015 ♀-mHFD: 0.569 ± 0.008	Diet: 14.00	Diet: 0.0016**
		Microglial density (cell/mm ²)	♂-CD: 6.560 ± 0.765 ♂-mHFD: 5.955 ± 0.343 ♀-CD: 6.454 ± 0.596 ♀-mHFD: 5.354 ± 0.729	Diet*Sex: 0.1449 Sex: 0.2945 Diet: 1.712	Diet*Sex: 0.7082 Sex: 0.5944 Diet: 0.2082
		NND (mm)	♂-CD: 24.70 ± 1.43 ♂-mHFD: 24.66 ± 0.63 ♀-CD: 24.61 ± 0.98 ♀-mHFD: 26.25 ± 1.49	Diet*Sex: 0.4768 Sex: 0.3725 Diet: 0.4323	Diet*Sex: 0.4992 Sex: 0.5497 Diet: 0.5196
		Spacing index (a.u.)	♂-CD: 0.369 ± 0.011 ♂-mHFD: 0.354 ± 0.005 ♀-CD: 0.377 ± 0.010 ♀-mHFD: 0.347 ± 0.012	Diet*Sex: 0.6695 Sex: 0.0006887 Diet: 5.341	Diet*Sex: 0.4245 Sex: 0.9794 Diet: 0.0336*
	Blood vessels	Vessel length (mm/mm ³)	♂-CD: 1.837 ± 0.040 ♂-mHFD: 2.128 ± 0.024 ♀-CD: 1.774 ± 0.072 ♀-mHFD: 1.990 ± 0.065	Diet*Sex: 0.4950 Sex: 3.676 Diet: 23.23	Diet*Sex: 0.4912 Sex: 0.0722 Diet: 0.0002***
		Number of branching points (/mm ³)	♂-CD: 3.225 ± 0.233 ♂-mHFD: 4.258 ± 0.144 ♀-CD: 3.705 ± 0.202 ♀-mHFD: 4.701 ± 0.309	Diet*Sex: 0.006343 Sex: 3.954 Diet: 19.13	Diet*Sex: 0.9374 Sex: 0.0631 Diet: 0.0004***
		Tortuosity index (a.u.)	♂-CD: 1.809 ± 0.053 ♂-mHFD: 1.866 ± 0.027 ♀-CD: 1.789 ± 0.003 ♀-mHFD: 1.745 ± 0.033	Diet*Sex: 1.811 Sex: 3.609 Diet: 0.02876	Diet*Sex: 0.1961 Sex: 0.0746 Diet: 0.8673
	Pericytes	Length coverage (mm)	♂-CD: 0.374 ± 0.072 ♂-mHFD: 0.338 ± 0.048 ♀-CD: 0.499 ± 0.114 ♀-mHFD: 0.343 ± 0.036	Diet*Sex: 0.6472 Sex: 0.7686 Diet: 1.684	Diet*Sex: 0.4322 Sex: 0.3929 Diet: 0.2117
		Volume coverage	♂-CD: 0.269 ± 0.053 ♂-mHFD: 0.248 ± 0.031 ♀-CD: 0.374 ± 0.087 ♀-mHFD: 0.252 ± 0.024	Diet*Sex: 0.8442 Sex: 0.9838 Diet: 1.718	Diet*Sex: 0.3711 Sex: 0.3352 Diet: 0.2074
	Microglia	Microglia density ratio (a.u.)	♂-CD: 0.924 ± 0.140 ♂-mHFD: 1.003 ± 0.058 ♀-CD: 1.091 ± 0.055 ♀-mHFD: 1.108 ± 0.095	Diet*Sex: 0.09519 Sex: 1.841 Diet: 0.2311	Diet*Sex: 0.7614 Sex: 0.1925 Diet: 0.6369
		Microglial nearest distance to blood vessel (μm)	♂-CD: 5.967 ± 0.212 ♂-mHFD: 5.433 ± 0.178 ♀-CD: 5.267 ± 0.263 ♀-mHFD: 4.700 ± 0.152	Diet*Sex: 0.006442 Sex: 11.91 Diet: 7.016	Diet*Sex: 0.9370 Sex: 0.0030** Diet: 0.0169*
			♂-CD: 0.509 ± 0.019	Diet*Sex: 0.1783	Diet*Sex: 0.6781

Occipital cortex		Ratio microglia in contact with blood vessel (a.u.)	♂-mHFD: 0.547 ± 0.015 ♀-CD: 0.555 ± 0.024 ♀-mHFD: 0.608 ± 0.013	Sex: 8.595 Diet: 6.041	Sex: 0.0093** Diet: 0.0250*
		Microglial density (cell/mm ²)	♂-CD: 6.199 ± 0.524 ♂-mHFD: 6.348 ± 0.345 ♀-CD: 6.583 ± 0.123 ♀-mHFD: 5.659 ± 0.517	Diet*Sex: 1.572 Sex: 0.1273 Diet: 0.8218	Diet*Sex: 0.2268 Sex: 0.7257 Diet: 0.3773
		NND (mm)	♂-CD: 25.25 ± 1.06 ♂-mHFD: 24.59 ± 0.58 ♀-CD: 23.66 ± 0.29 ♀-mHFD: 25.56 ± 0.92	Diet*Sex: 2.453 Sex: 0.1451 Diet: 0.5775	Diet*Sex: 0.1357 Sex: 0.7080 Diet: 0.4577
		Spacing index (a.u.)	♂-CD: 0.380 ± 0.007 ♂-mHFD: 0.375 ± 0.005 ♀-CD: 0.367 ± 0.005 ♀-mHFD: 0.361 ± 0.007	Diet*Sex: 0.009025 Sex: 4.948 Diet: 0.9504	Diet*Sex: 0.9254 Sex: 0.0399* Diet: 0.3433
	Blood vessels	Vessel length (mm/mm ³)	♂-CD: 1.489 ± 0.094 ♂-mHFD: 1.846 ± 0.083 ♀-CD: 1.472 ± 0.077 ♀-mHFD: 1.555 ± 0.046	Diet*Sex: 2.959 Sex: 3.746 Diet: 7.681	Diet*Sex: 0.1036 Sex: 0.0697 Diet: 0.0131*
		Number of branching points (/mm ³)	♂-CD: 2.470 ± 0.278 ♂-mHFD: 3.437 ± 0.218 ♀-CD: 2.814 ± 0.187 ♀-mHFD: 3.170 ± 0.216	Diet*Sex: 1.689 Sex: 0.02679 Diet: 7.929	Diet*Sex: 0.2110 Sex: 0.8719 Diet: 0.0119*
		Tortuosity index (a.u.)	♂-CD: 1.754 ± 0.040 ♂-mHFD: 1.791 ± 0.059 ♀-CD: 1.774 ± 0.031 ♀-mHFD: 1.830 ± 0.052	Diet*Sex: 0.04131 Sex: 0.4022 Diet: 1.018	Diet*Sex: 0.8414 Sex: 0.5344 Diet: 0.3271
	Pericytes	Length coverage (mm)	♂-CD: 0.419 ± 0.073 ♂-mHFD: 0.357 ± 0.044 ♀-CD: 0.481 ± 0.091 ♀-mHFD: 0.407 ± 0.063	Diet*Sex: 0.006198 Sex: 0.6348 Diet: 0.9225	Diet*Sex: 0.9382 Sex: 0.4366 Diet: 0.3503
		Volume coverage	♂-CD: 0.304 ± 0.054 ♂-mHFD: 0.250 ± 0.030 ♀-CD: 0.359 ± 0.069 ♀-mHFD: 0.297 ± 0.044	Diet*Sex: 0.005284 Sex: 0.9421 Diet: 1.255	Diet*Sex: 0.9429 Sex: 0.3453 Diet: 0.2782
	Microglia	Microglia density ratio (a.u.)	♂-CD: 0.911 ± 0.149 ♂-mHFD: 1.095 ± 0.077 ♀-CD: 1.106 ± 0.053 ♀-mHFD: 1.092 ± 0.115	Diet*Sex: 0.7910 Sex: 0.7425 Diet: 0.5711	Diet*Sex: 0.3862 Sex: 0.4008 Diet: 0.4602
		Microglial nearest distance to blood vessel (μm)	♂-CD: 7.389 ± 0.384 ♂-mHFD: 6.640 ± 0.137 ♀-CD: 6.380 ± 0.359 ♀-mHFD: 5.900 ± 0.136	Diet*Sex: 0.2072 Sex: 8.763 Diet: 4.327	Diet*Sex: 0.6548 Sex: 0.0088** Diet: 0.0530

Hippocampus		Ratio microglia in contact with blood vessel (a.u.)	♂-CD: 0.432 ± 0.020 ♂-mHFD: 0.463 ± 0.006 ♀-CD: 0.492 ± 0.021 ♀-mHFD: 0.524 ± 0.016	<i>Diet*Sex</i> : 0.0008377 <i>Sex</i> : 12.40 <i>Diet</i> : 3.325	<i>Diet*Sex</i> : 0.9772 <i>Sex</i> : 0.0026** <i>Diet</i> : 0.0859
		Microglial density (cell/mm ²)	♂-CD: 6.867 ± 0.638 ♂-mHFD: 7.742 ± 0.328 ♀-CD: 7.451 ± 0.284 ♀-mHFD: 6.903 ± 0.783	<i>Diet*Sex</i> : 1.590 <i>Sex</i> : 0.05123 <i>Diet</i> : 0.08403	<i>Diet*Sex</i> : 0.2244 <i>Sex</i> : 0.8236 <i>Diet</i> : 0.7754
		NND (mm)	♂-CD: 24.63 ± 1.64 ♂-mHFD: 22.38 ± 0.42 ♀-CD: 22.72 ± 0.40 ♀-mHFD: 24.04 ± 1.23	<i>Diet*Sex</i> : 2.414 <i>Sex</i> : 0.01126 <i>Diet</i> : 0.1599	<i>Diet*Sex</i> : 0.1387 <i>Sex</i> : 0.9167 <i>Diet</i> : 0.6942
		Spacing index (a.u.)	♂-CD: 0.381 ± 0.005 ♂-mHFD: 0.382 ± 0.006 ♀-CD: 0.377 ± 0.005 ♀-mHFD: 0.383 ± 0.012	<i>Diet*Sex</i> : 0.4983 <i>Sex</i> : 0.4345 <i>Diet</i> : 0.0005275	<i>Diet*Sex</i> : 0.4898 <i>Sex</i> : 0.5186 <i>Diet</i> : 0.9819
	Blood vessels	Vessel length (mm/mm ³)	♂-CD: 1.383 ± 1.102 ♂-mHFD: 1.646 ± 0.061 ♀-CD: 1.518 ± 0.205 ♀-mHFD: 1.275 ± 0.042	<i>Diet*Sex</i> : 4.480 <i>Sex</i> : 0.9751 <i>Diet</i> : 0.007500	<i>Diet*Sex</i> : 0.0493* <i>Sex</i> : 0.3373 <i>Diet</i> : 0.9320
		Number of branching points (/mm ³)	♂-CD: 2.238 ± 0.245 ♂-mHFD: 3.178 ± 0.270 ♀-CD: 2.886 ± 0.516 ♀-mHFD: 2.236 ± 0.169	<i>Diet*Sex</i> : 6.118 <i>Sex</i> : 0.2087 <i>Diet</i> : 0.2047	<i>Diet*Sex</i> : 0.0242* <i>Sex</i> : 0.6536 <i>Diet</i> : 0.6567
		Tortuosity index (a.u.)	♂-CD: 1.800 ± 0.030 ♂-mHFD: 1.958 ± 0.037 ♀-CD: 1.848 ± 0.056 ♀-mHFD: 1.856 ± 0.075	<i>Diet*Sex</i> : 2.145 <i>Sex</i> : 0.2770 <i>Diet</i> : 2.664	<i>Diet*Sex</i> : 0.1613 <i>Sex</i> : 0.6055 <i>Diet</i> : 0.1210
	Pericytes	Length coverage (mm)	♂-CD: 0.401 ± 0.058 ♂-mHFD: 0.368 ± 0.048 ♀-CD: 0.410 ± 0.077 ♀-mHFD: 0.474 ± 0.041	<i>Diet*Sex</i> : 0.7118 <i>Sex</i> : 0.9734 <i>Diet</i> : 0.07137	<i>Diet*Sex</i> : 0.4106 <i>Sex</i> : 0.3377 <i>Diet</i> : 0.7926
		Volume coverage	♂-CD: 0.313 ± 0.044 ♂-mHFD: 0.273 ± 0.034 ♀-CD: 0.314 ± 0.052 ♀-mHFD: 0.373 ± 0.027	<i>Diet*Sex</i> : 1.443 <i>Sex</i> : 1.553 <i>Diet</i> : 0.05501	<i>Diet*Sex</i> : 0.2461 <i>Sex</i> : 0.2296 <i>Diet</i> : 0.8174
	Microglia	Microglia density ratio (a.u.)	♂-CD: 0.836 ± 0.099 ♂-mHFD: 1.064 ± 0.023 ♀-CD: 1.185 ± 0.046 ♀-mHFD: 0.932 ± 0.153	<i>Diet*Sex</i> : 6.315 <i>Sex</i> : 1.294 <i>Diet</i> : 0.01648	<i>Diet*Sex</i> : 0.0223* <i>Sex</i> : 0.2711 <i>Diet</i> : 0.8994
		Microglial nearest distance to blood vessel (μm)	♂-CD: 8.900 ± 0.313 ♂-mHFD: 7.650 ± 0.439 ♀-CD: 7.067 ± 0.435	<i>Diet*Sex</i> : 5.674 <i>Sex</i> : 5.799 <i>Diet</i> : 0.7814	<i>Diet*Sex</i> : 0.0292* <i>Sex</i> : 0.0277* <i>Diet</i> : 0.3890

			♀-mHFD: 7.640 ± 0.346		
	Ratio microglia in contact with blood vessel (a.u.)	♂-CD: 0.397 ± 0.017 ♂-mHFD: 0.460 ± 0.019 ♀-CD: 0.481 ± 0.020 ♀-mHFD: 0.462 ± 0.018	Diet*Sex: 4.878 Sex: 5.361 Diet: 1.382	Diet*Sex: 0.0412* Sex: 0.0333* Diet: 0.2560	
	Microglial density (cell/mm ²)	♂-CD: 5.925 ± 0.453 ♂-mHFD: 6.706 ± 0.191 ♀-CD: 6.638 ± 0.085 ♀-mHFD: 5.312 ± 0.636	Diet*Sex: 6.519 Sex: 0.6792 Diet: 0.4359	Diet*Sex: 0.0206* Sex: 0.4213 Diet: 0.5180	
	NND (mm)	♂-CD: 25.34 ± 0.88 ♂-mHFD: 23.14 ± 0.37 ♀-CD: 23.48 ± 0.32 ♀-mHFD: 26.67 ± 1.66	Diet*Sex: 7.725 Sex: 0.7392 Diet: 0.2669	Diet*Sex: 0.0128* Sex: 0.4019 Diet: 0.6121	
	Spacing index (a.u.)	♂-CD: 0.372 ± 0.009 ♂-mHFD: 0.358 ± 0.005 ♀-CD: 0.363 ± 0.006 ♀-mHFD: 0.360 ± 0.008	Diet*Sex: 0.5688 Sex: 0.1806 Diet: 1.399	Diet*Sex: 0.4610 Sex: 0.6762 Diet: 0.2532	

Supplementary Table 2. Summary of Mixed-effect models test to assess *Diet* and *Sex* main effects as well as *Diet*Sex* interaction effect of ultrastructure analysis of capillaries. Data are presented as Mean ± SEM for each treatment group. *F* and *P* values are given for main and interactions effects (n=93-95 capillaries/diet/sex). ♂: male, ♀: female, a.u.: arbitrary unit, CD: control diet, mHFD: maternal high-fat diet, SEM: standard error of the mean.

Parameters		Mean ± SEM	<i>F</i>	<i>P</i>
Endothelium	Area (μm ²)	♂-CD: 5.784±0.359 ♂-mHFD: 5.830±0.332 ♀-CD: 5.656±0.376 ♀-mHFD: 5.295±0.293	Diet*Sex: 0.3538 Sex: 0.9429 Diet: 0.2114	Diet*Sex: 0.5523 Sex: 0.3322 Diet: 0.6459
	Basal membrane thickness (nm)	♂-CD: 82.57±2.32 ♂-mHFD: 75.89±2.25 ♀-CD: 76.24±2.13 ♀-mHFD: 76.04±2.12	Diet*Sex: 2.156 Sex: 1.961 Diet: 2.426	Diet*Sex: 0.1428 Sex: 0.1623 Diet: 0.1202
	# mitochondria per endothelium volume (/μm ²)	♂-CD: 0.665±0.043 ♂-mHFD: 0.674±0.053 ♀-CD: 0.658±0.056	Diet*Sex: 0.03531 Sex: 0.0008057 Diet: 0.1399	Diet*Sex: 0.8511 Sex: 0.9774 Diet: 0.7086

		♀-mHFD: 0.685±0.042		
	Ratio of mitochondrial fission/fusion (a.u.)	♂-CD: 0.034±0.010 ♂-mHFD: 0.023±0.017 ♀-CD: 0.028±0.010 ♀-mHFD: 0.017±0.011	Diet*Sex: 2.719e-6 Sex: 0.2000 Diet: 0.8210	Diet*Sex: 0.9987 Sex: 0.6550 Diet: 0.3655
	# tight junctions per endothelium volume (/μm ²)	♂-CD: 0.092±0.007 ♂-mHFD: 0.082±0.007 ♀-CD: 0.076±0.006 ♀-mHFD: 0.063±0.006	Diet*Sex: 0.04699 Sex: 6.954 Diet: 3.196	Diet*Sex: 0.8285 Sex: 0.0087** Diet: 0.0746
	Average length of tight junctions (nm)	♂-CD: 736.3±49.9 ♂-mHFD: 793.2±61.9 ♀-CD: 782.8±57.6 ♀-mHFD: 773.4±63.6	Diet*Sex: 0.3193 Sex: 0.05137 Diet: 0.1637	Diet*Sex: 0.5727 Sex: 0.8209 Diet: 0.6862
	Average thickness of tight junctions (nm)	♂-CD: 176.7±7.1 ♂-mHFD: 181.5±8.0 ♀-CD: 177.2±8.0 ♀-mHFD: 174.9±7.7	Diet*Sex: 0.2116 Sex: 0.1524 Diet: 0.02316	Diet*Sex: 0.6461 Sex: 0.6967 Diet: 0.8792
Pericyte	# pericytic process per basal membrane length (/μm)	♂-CD: 0.152±0.011 ♂-mHFD: 0.155±0.008 ♀-CD: 0.141±0.010 ♀-mHFD: 0.132±0.007	Diet*Sex: 0.4568 Sex: 3.550 Diet: 0.1232	Diet*Sex: 0.4996 Sex: 0.0603 Diet: 0.7258
	# of lipidic inclusion per pericytic process	♂-CD: 0.011±0.011 ♂-mHFD: 0.000±0.000 ♀-CD: 0.011±0.011 ♀-mHFD: 0.032±0.024	Diet*Sex: 1.324 Sex: 1.306 Diet: 0.1555	Diet*Sex: 0.2507 Sex: 0.2539 Diet: 0.6936
Other perivascular cell	# of perivascular cellular process per endothelium length (/μm)	♂-CD: 0.007±0.003 ♂-mHFD: 0.008±0.003 ♀-CD: 0.004±0.002 ♀-mHFD: 0.013±0.005	Diet*Sex: 1.395 Sex: 0.06352 Diet: 2.722	Diet*Sex: 0.2384 Sex: 0.8012 Diet: 0.0998
	# of lipidic inclusion per perivascular cellular process	♂-CD: 0.043±0.043 ♂-mHFD: 0.140±0.140 ♀-CD: 0.000±0.000	Diet*Sex: 0.1326 Sex: 0.8752 Diet: 0.8868	Diet*Sex: 0.7160 Sex: 0.3501 Diet: 0.3470

		♀-mHFD: 0.043±0.034		
Astrocyte	# astrocytic endfeet per endothelium length (/μm)	♂-CD: 0.314±0.014 ♂-mHFD: 0.293±0.016 ♀-CD: 0.309±0.017 ♀-mHFD: 0.292±0.015	<i>Diet*Sex</i> : 0.02136 <i>Sex</i> : 0.04319 <i>Diet</i> : 1.446	<i>Diet*Sex</i> : 0.8839 <i>Sex</i> : 0.8355 <i>Diet</i> : 0.2300
	Average area of astrocytic endfeet (μm ²)	♂-CD: 2.324±0.263 ♂-mHFD: 1.569±0.155 ♀-CD: 1.971±0.209 ♀-mHFD: 2.261±0.287	<i>Diet*Sex</i> : 4.992 <i>Sex</i> : 0.5213 <i>Diet</i> : 0.9882	<i>Diet*Sex</i>: 0.0261* <i>Sex</i> : 0.4707 <i>Diet</i> : 0.3208
	Ratio of astrocytic endfeet containing glycogen granules (a.u.)	♂-CD: 0.075±0.028 ♂-mHFD: 0.247±0.045 ♀-CD: 0.179±0.040 ♀-mHFD: 0.151±0.037	<i>Diet*Sex</i> : 7.001 <i>Sex</i> : 0.08306 <i>Diet</i> : 3.594	<i>Diet*Sex</i>: 0.0085** <i>Sex</i> : 0.9274 <i>Diet</i> : 0.0588
Microglia	Ratio of microglia in direct contact with the basal membrane	♂-CD: 0.167±0.041 ♂-mHFD: 0.075±0.030 ♀-CD: 0.143±0.040 ♀-mHFD: 0.086±0.031	<i>Diet*Sex</i> : 0.2402 <i>Sex</i> : 0.02970 <i>Diet</i> : 4.245	<i>Diet*Sex</i> : 0.6244 <i>Sex</i> : 0.8633 <i>Diet</i>: 0.0402*

Supplementary Table 3. Summary of Mixed-effect models test to assess *Diet* and *Sex* main effects as well as *Diet*Sex* interaction effect of ultrastructure analysis of microglia. Data are presented as Mean ± SEM for each treatment group. *F* and *P* values are given for main and interactions effects (n=77-84 microglia/diet/sex). ♂: male, ♀: female, a.u.: arbitrary unit, CD: control diet, mHFD: maternal high-fat diet, SEM: standard error of the mean.

Parameters		Mean ± SEM	<i>F</i>	<i>P</i>
Phagolysosomal	# primary lysosomes per microglia	♂-CD: 0.893±0.171	<i>Diet*Sex</i> : 1.585	<i>Diet*Sex</i> : 0.2090
		♂-mHFD: 1.275±0.176	<i>Sex</i> : 0.2976	<i>Sex</i> : 0.5858
		♀-CD: 1.026±0.200	<i>Diet</i> : 0.6224	<i>Diet</i> : 0.4308
		♀-mHFD: 0.938±0.199		

	# secondary lysosomes per microglia	♂-CD: 0.143±0.042 ♂-mHFD: 0.163±0.045 ♀-CD: 0.156±0.049 ♀-mHFD: 0.358±0.088	Diet*Sex: 2.369 Sex: 3.091 Diet: 3.499	Diet*Sex: 0.1247 Sex: 0.0797 Diet: 0.0623
	# tertiary lysosomes per microglia	♂-CD: 0.190±0.071 ♂-mHFD: 0.138±0.050 ♀-CD: 0.039±0.022 ♀-mHFD: 0.062±0.027	Diet*Sex: 0.6323 Sex: 5.693 Diet: 0.1006	Diet*Sex: 0.1153 Sex: 0.0176* Diet: 0.7514
	# lipofuscin granules per microglia	♂-CD: 0.107±0.034 ♂-mHFD: 0.088±0.032 ♀-CD: 0.065±0.028 ♀-mHFD: 0.062±0.027	Diet*Sex: 0.07260 Sex: 1.242 Diet: 0.1403	Diet*Sex: 0.7878 Sex: 0.2659 Diet: 0.7082
	# empty endosomes per microglia	♂-CD: 0.095±0.036 ♂-mHFD: 0.025±0.018 ♀-CD: 0.130±0.043 ♀-mHFD: 0.185±0.050	Diet*Sex: 2.644 Sex: 6.367 Diet: 0.03736	Diet*Sex: 0.1049 Sex: 0.0121* Diet: 0.8469
	# endosomes with digested content per microglia	♂-CD: 0.298±0.067 ♂-mHFD: 0.350±0.102 ♀-CD: 0.377±0.072 ♀-mHFD: 0.531±0.108	Diet*Sex: 0.3252 Sex: 2.116 Diet: 1.338	Diet*Sex: 0.5689 Sex: 0.1467 Diet: 0.2483
Metabolism	# mitochondria per microglia	♂-CD: 2.107±0.186 ♂-mHFD: 3.488±0.351 ♀-CD: 2.675±0.273 ♀-mHFD: 2.901±0.293	Diet*Sex: 4.239 Sex: 0.001040 Diet: 8.206	Diet*Sex: 0.0403* Sex: 0.9743 Diet: 0.0045**
	# lipidic bodies per microglia	♂-CD: 0.167±0.050 ♂-mHFD: 0.050±0.025 ♀-CD: 0.013±0.013 ♀-mHFD: 0.037±0.021	Diet*Sex: 4.996 Sex: 7.006 Diet: 2.164	Diet*Sex: 0.0261* Sex: 0.0085** Diet: 0.1423
Cellular	# mitochondrial anomalies per microglia	♂-CD: 0.440±0.075 ♂-mHFD: 0.600±0.113	Diet*Sex: 0.02332 Sex: 2.632	Diet*Sex: 0.8787 Sex: 0.1057

		♀-CD: 0.312±0.079 ♀-mHFD: 0.444±0.079	Diet: 2.781	Diet: 0.0964
	Ratio with dilated cisternae of endoplasmic reticulum and Golgi apparatus (a.u.)	♂-CD: 0.929±0.028 ♂-mHFD: 0.888±0.036 ♀-CD: 0.792±0.047 ♀-mHFD: 0.938±0.027	Diet*Sex: 7.240 Sex: 1.515 Diet: 2.279	Diet*Sex: 0.0075** Sex: 0.2194 Diet: 0.1321
Interaction with their microenvironment	# contacts with presynaptic terminal	♂-CD: 4.774±0.327 ♂-mHFD: 6.925±0.375 ♀-CD: 5.156±0.336 ♀-mHFD: 4.679±0.320	Diet*Sex: 14.92 Sex: 7.504 Diet: 6.055	Diet*Sex: 0.0001*** Sex: 0.0065** Diet: 0.0144*
	# contacts with postsynaptic dendrites	♂-CD: 2.583±0.220 ♂-mHFD: 3.388±0.256 ♀-CD: 2.857±0.240 ♀-mHFD: 2.432±0.206	Diet*Sex: 7.068 Sex: 2.173 Diet: 0.6724	Diet*Sex: 0.0082** Sex: 0.1414 Diet: 0.4128
	# contacts with myelinated axons	♂-CD: 0.333±0.068 ♂-mHFD: 0.225±0.067 ♀-CD: 0.260±0.068 ♀-mHFD: 0.185±0.050	Diet*Sex: 0.07038 Sex: 0.7934 Diet: 2.063	Diet*Sex: 0.7910 Sex: 0.3738 Diet: 0.1519
	# contacts with degraded myelin	♂-CD: 0.145±0.042 ♂-mHFD: 0.075±0.035 ♀-CD: 0.117±0.049 ♀-mHFD: 0.185±0.050	Diet*Sex: 2.417 Sex: 0.8651 Diet: 0.0002071	Diet*Sex: 0.1210 Sex: 0.3530 Diet: 0.9885
	Ratio touching a neuronal cell (a.u.)	♂-CD: 0.321±0.051 ♂-mHFD: 0.325±0.053 ♀-CD: 0.312±0.059 ♀-mHFD: 0.519±0.056	Diet*Sex: 3.448 Sex: 2.819 Diet: 3.694	Diet*Sex: 0.0643 Sex: 0.0942 Diet: 0.0555
	Ratio touching an astrocytic cell (a.u.)	♂-CD: 0.024±0.017 ♂-mHFD: 0.025±0.018 ♀-CD: 0.039±0.022 ♀-mHFD: 0.000±0.000	Diet*Sex: 1.519 Sex: 0.09139 Diet: 1.344	Diet*Sex: 0.2187 Sex: 0.7676 Diet: 0.2472

	Ratio touching an oligodendrocytic cell (a.u.)	♂-CD: 0.012±0.012 ♂-mHFD: 0.000±0.000 ♀-CD: 0.026±0.018 ♀-mHFD: 0.012±0.012	<i>Diet*Sex</i> : 0.004841 <i>Sex</i> : 1.137 <i>Diet</i> : 1.062	<i>Diet*Sex</i> : 0.9446 <i>Sex</i> : 0.2871 <i>Diet</i> : 0.3035
	# microglia-associated extracellular space pockets	♂-CD: 5.298±0.340 ♂-mHFD: 3.213±0.270 ♀-CD: 5.221±0.365 ♀-mHFD: 4.926±0.287	<i>Diet*Sex</i> : 7.931 <i>Sex</i> : 6.628 <i>Diet</i> : 14.02	<i>Diet*Sex</i>: 0.0052** <i>Sex</i>: 0.0105* <i>Diet</i>: 0.0002***
	# exophagy events per microglia	♂-CD: 0.369±0.067 ♂-mHFD: 0.225±0.050 ♀-CD: 0.182±0.048 ♀-mHFD: 0.420±0.080	<i>Diet*Sex</i> : 9.084 <i>Sex</i> : 0.003524 <i>Diet</i> : 0.5488	<i>Diet*Sex</i>: 0.0028** <i>Sex</i> : 0.9527 <i>Diet</i> : 0.4594

Supplementary Table 4. Summary of ANOVAs test to assess *Diet* and *Sex* main effects as well as *Diet*Sex* interaction effect of behavioural data. Data are presented as Mean ± SEM for each treatment group. *F* and *P* values are given for main and interactions effects (n=6-10 litters/diet/sex, N=8-12 animals/group). ♂: male, ♀: female, a.u.: arbitrary unit, CD: control diet, mHFD: maternal high-fat diet, SEM: standard error of the mean.

Parameters		Mean ± SEM	<i>F</i>	<i>P</i>
Marbles burying	Number of buried marbles	♂-CD: 5.055 ± 1.320	<i>Diet*Sex</i> : 0.3047	<i>Diet*Sex</i> : 0.5852
		♂-mHFD: 9.111 ± 0.553	<i>Sex</i> : 1.555	<i>Sex</i> : 0.2224
		♀-CD: 4.291 ± 1.493	<i>Diet</i> : 9.852	<i>Diet</i> : 0.0039**
		♀-mHFD: 7.134 ± 0.939		
Open field	Distance travelled (cm)	♂-CD: 995.6 ± 66.9	<i>Diet*Sex</i> : 0.7673	<i>Diet*Sex</i> : 0.3866
		♂-mHFD: 1167.2 ± 135.9	<i>Sex</i> : 6.241	<i>Sex</i> : 0.0169*
		♀-CD: 1403.6 ± 139.0	<i>Diet</i> : 0.2946	<i>Diet</i> : 0.5905
		♀-mHFD: 1363.3 ± 125.8		
	Moving time (min)	♂-CD: 1.795 ± 0.105 ♂-mHFD: 2.216 ± 0.242 ♀-CD: 2.556 ± 0.212 ♀-mHFD: 2.436 ± 0.226	<i>Diet*Sex</i> : 1.688 <i>Sex</i> : 5.545 <i>Diet</i> : 0.5216	<i>Diet*Sex</i> : 0.2016 <i>Sex</i> : 0.0238* <i>Diet</i> : 0.4746
	Center time (min)	♂-CD: 1.946 ± 0.311	<i>Diet*Sex</i> : 3.793	<i>Diet*Sex</i> : 0.0589

		♂-mHFD: 2.865 ± 0.349 ♀-CD: 2.781 ± 0.438 ♀-mHFD: 2.325 ± 0.323	Sex: 0.1745 Diet: 0.4304	Sex: 0.6785 Diet: 0.5157
	Number of revolutions	♂-CD: 9.375 ± 1.194 ♂-mHFD: 6.545 ± 0.755 ♀-CD: 9.417 ± 1.151 ♀-mHFD: 8.818 ± 1.577	Diet*Sex: 0.8201 Sex: 0.8825 Diet: 1.936	Diet*Sex: 0.3709 Sex: 0.3534 Diet: 0.1722
	Number of stereotypies	♂-CD: 10.47 ± 0.25 ♂-mHFD: 10.68 ± 0.23 ♀-CD: 10.88 ± 0.24 ♀-mHFD: 10.92 ± 0.15	Diet*Sex: 0.1445 Sex: 2.093 Diet: 0.3242	Diet*Sex: 0.7059 Sex: 0.1562 Diet: 0.5724
	Number of stereotypies count	♂-CD: 178.1 ± 17.1 ♂-mHFD: 192.8 ± 17.1 ♀-CD: 218.1 ± 13.4 ♀-mHFD: 192.6 ± 14.7	Diet*Sex: 1.521 Sex: 1.488 Diet: 0.1092	Diet*Sex: 0.2250 Sex: 0.2300 Diet: 0.7429
	Duration of stereotypies	♂-CD: 3.067 ± 0.258 ♂-mHFD: 3.112 ± 0.228 ♀-CD: 3.265 ± 0.214 ♀-mHFD: 2.922 ± 0.155	Diet*Sex: 0.7801 Sex: 0.0002930 Diet: 0.4599	Diet*Sex: 0.3827 Sex: 0.9864 Diet: 0.5018
Elevated plus-maze	Time spent in closed arms (sec)	♂-CD: 206.2 ± 11.8 ♂-mHFD: 196.8 ± 9.9 ♀-CD: 206.4 ± 5.3 ♀-mHFD: 203.1 ± 7.9	Diet*Sex: 0.1104 Sex: 0.1220 Diet: 0.4802	Diet*Sex: 0.7421 Sex: 0.7294 Diet: 0.4938
	Time spent in open arms (sec)	♂-CD: 32.83 ± 7.73 ♂-mHFD: 61.01 ± 24.42 ♀-CD: 40.12 ± 6.47 ♀-mHFD: 48.43 ± 17.16	Diet*Sex: 0.3683 Sex: 0.02612 Diet: 1.240	Diet*Sex: 0.5482 Sex: 0.8726 Diet: 0.2737
	Anxiety index (a.u.)	♂-CD: 0.113 ± 0.038 ♂-mHFD: 0.143 ± 0.033 ♀-CD: 0.135 ± 0.030 ♀-mHFD: 0.166 ± 0.026	Diet*Sex: 0.0001405 Sex: 0.4857 Diet: 0.8940	Diet*Sex: 0.9906 Sex: 0.4914 Diet: 0.3522
Three-chambers test	Social preference (a.u.)	♂-CD: 1.150 ± 0.127 ♂-mHFD: 1.256 ± 0.166 ♀-CD: 1.270 ± 0.187 ♀-mHFD: 1.159 ± 0.126	Diet*Sex: 0.4808 Sex: 0.005218 Diet: 0.0002941	Diet*Sex: 0.4942 Sex: 0.9430 Diet: 0.9864
	Social novelty preference (a.u.)	♂-CD: 1.104 ± 0.160 ♂-mHFD: 1.096 ± 0.253 ♀-CD: 1.142 ± 0.260 ♀-mHFD: 1.196 ± 0.198	Diet*Sex: 0.01696 Sex: 0.08394 Diet: 0.009322	Diet*Sex: 0.8974 Sex: 0.7744 Diet: 0.9239
Novel object recogn	Acquisition: exploration time (sec)	♂-CD: 53.81 ± 4.49 ♂-mHFD: 50.29 ± 4.53	Diet*Sex: 0.0006174 Sex: 0.2768	Diet*Sex: 0.9804 Sex: 0.6029

		♀-CD: 55.81 ± 5.05 ♀-mHFD: 52.49 ± 2.41	<i>Diet</i> : 0.7361	<i>Diet</i> : 0.3982
	Recognition: exploration time (sec)	♂-CD: 62.79 ± 3.46 ♂-mHFD: 61.88 ± 2.19 ♀-CD: 68.13 ± 5.40 ♀-mHFD: 66.85 ± 1.87	<i>Diet*Sex</i> : 0.003415 <i>Sex</i> : 2.438 <i>Diet</i> : 0.1097	<i>Diet*Sex</i> : 0.9538 <i>Sex</i> : 0.1301 <i>Diet</i> : 0.7430
Prepulse inhibition	PP3: % of inhibition	♂-CD: 27.90 ± 6.10 ♂-mHFD: 24.88 ± 3.84 ♀-CD: 30.96 ± 3.56 ♀-mHFD: 22.34 ± 5.85	<i>Diet*Sex</i> : 0.3772 <i>Sex</i> : 2.825 <i>Diet</i> : 0.1567	<i>Diet*Sex</i> : 0.5434 <i>Sex</i> : 0.1026 <i>Diet</i> : 0.6948
	PP6: % of inhibition	♂-CD: 40.42 ± 5.29 ♂-mHFD: 44.52 ± 6.46 ♀-CD: 44.56 ± 5.19 ♀-mHFD: 49.12 ± 3.71	<i>Diet*Sex</i> : 0.08449 <i>Sex</i> : 0.1398 <i>Diet</i> : 0.1351	<i>Diet*Sex</i> : 0.7732 <i>Sex</i> : 0.7110 <i>Diet</i> : 0.7157
	PP9: % of inhibition	♂-CD: 52.43 ± 5.23 ♂-mHFD: 50.51 ± 6.64 ♀-CD: 49.86 ± 5.85 ♀-mHFD: 48.69 ± 6.45	<i>Diet*Sex</i> : 0.1058 <i>Sex</i> : 0.1709 <i>Diet</i> : 0.09708	<i>Diet*Sex</i> : 0.7471 <i>Sex</i> : 0.6820 <i>Diet</i> : 0.7574
	PP12: % of inhibition	♂-CD: 44.11 ± 7.26 ♂-mHFD: 62.64 ± 4.29 ♀-CD: 56.82 ± 5.88 ♀-mHFD: 58.07 ± 4.63	<i>Diet*Sex</i> : 1.300 <i>Sex</i> : 0.3694 <i>Diet</i> : 2.414	<i>Diet*Sex</i> : 0.2627 <i>Sex</i> : 0.5476 <i>Diet</i> : 0.1301
	PP15: % of inhibition	♂-CD: 59.34 ± 5.48 ♂-mHFD: 56.30 ± 6.84 ♀-CD: 63.63 ± 4.33 ♀-mHFD: 68.30 ± 3.85	<i>Diet*Sex</i> : 1.413 <i>Sex</i> : 2.102 <i>Diet</i> : 0.008190	<i>Diet*Sex</i> : 0.2433 <i>Sex</i> : 0.1568 <i>Diet</i> : 0.9285
	Mean: % of inhibition	♂-CD: 49.08 ± 5.24 ♂-mHFD: 53.49 ± 5.41 ♀-CD: 53.72 ± 4.11 ♀-mHFD: 54.69 ± 4.04	<i>Diet*Sex</i> : 0.1292 <i>Sex</i> : 0.3696 <i>Diet</i> : 0.3148	<i>Diet*Sex</i> : 0.7214 <i>Sex</i> : 0.5471 <i>Diet</i> : 0.5783
	Amplitude of startle response (a.u.)	♂-CD: 48.41 ± 6.19 ♂-mHFD: 66.70 ± 12.65 ♀-CD: 55.59 ± 7.77 ♀-mHFD: 67.44 ± 10.54	<i>Diet*Sex</i> : 0.1168 <i>Sex</i> : 0.1766 <i>Diet</i> : 2.558	<i>Diet*Sex</i> : 0.7346 <i>Sex</i> : 0.6769 <i>Diet</i> : 0.1187

Supplementary Table 5. Detailed diet composition of control chow vs high-fat diet. The different components of the diet are presented as either a percentage of food or a quantity (g, mg or µg) per kilogram of food. CD: control diet, HFD: high-fat diet, N/A: not available. ¹ Mineral mix AIN-93G-MX (cat# 94046, Teklad), ² Vitamins mix AIN-93-VX (cat# 94047, Teklad).

	units	CD	HFD
Macronutrients			
Crude protein	%	14.3	23.4
Fats	%	4	34.3
Lard	g/kg	N/A	310
Soybean Oil	g/kg	N/A	30
Cellulose	g/kg	N/A	65.5
Carbohydrate	%	48	27.3
Crude fiber	%	4.1	N/A
Neutral detergent fiber	%	18	N/A
Ash	%	4.7	N/A
Mineral			48¹
Calcium	%	0.7	N/A
Phosphorus	%	0.6	N/A
Sodium	%	0.1	N/A
Potassium	%	0.6	N/A
Chloride	%	0.3	N/A
Magnesium	%	0.2	N/A
Zinc	mg/kg	70	N/A
Manganese	mg/kg	100	N/A
Cooper	mg/kg	15	N/A
Iodine	mg/kg	6	N/A
Iron	mg/kg	175	N/A
Selenium	mg/kg	0.23	N/A
Calcium phosphate, dibasic	g/kg	-	3.4
Amino acids			
Aspartic acid	%	0.9	N/A
Glutamic acid	%	2.9	N/A
Alanine	%	0.9	N/A
Glycine	%	0.7	N/A
Threonine	%	0.5	N/A
Proline	%	1.2	N/A
Serine	%	0.7	N/A
Leucine	%	1.4	N/A
Isoleucine	%	0.6	N/A
Valine	%	0.7	N/A
Phenylalanine	%	0.7	N/A
Tyrosine	%	0.4	N/A
Methionine	%	0.3	N/A
Cystine	%	0.3	0.04
Lysine	%	0.7	N/A
Histidine	%	0.4	N/A
Arginine	%	0.8	N/A
Tryptophan	%	0.2	N/A

Vitamins			21²
Vitamin A	mg/kg	1.8	N/A
Vitamin D3	µg/kg	15	N/A
Vitamin E	mg/kg	80.4	N/A
Vitamin K	mg/kg	20	N/A
Vitamin B1 (thiamin)	mg/kg	12	N/A
Vitamin B2 (riboflavin)	mg/kg	6	N/A
Niacin (nicotinic acid)	mg/kg	54	N/A
Vitamin B6 (pyridoxine)	mg/kg	10	N/A
Pantothenic acid	mg/kg	17	N/A
Vitamin B12 (cyanocobalamin)	mg/kg	0.03	N/A
Biotin	mg/kg	0.26	N/A
Folate	mg/kg	2	N/A
Choline	mg/kg	1030	3000 * <i>choline bitartrate</i>
Fatty acids			
C16:0 Palmitic	%	0.5	N/A
C18:0 Stearic	%	0.1	N/A
C18:1ω9 Oleic	%	0.7	N/A
C18:2ω6 Linoleic	%	2	N/A
C18:3ω3 Linolenic	%	0.1	N/A
Total saturated	%	0.6	36
Total monounsaturated	%	0.7	41
Total polyunsaturated	%	2.1	23
Other			
Blue food color	g/kg	-	0.1
Casein	g/kg	-	265
Maltodextrin	g/kg	-	160
Sucrose	g/kg	-	90