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ARTICLE

Maternal dietary omega-3 deficiency worsens the deleterious effects of prenatal inflammation on the gut-brain axis in the offspring across lifetime

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Maternal immune activation (MIA) and poor maternal nutritional habits are risk factors for the occurrence of neurodevelopmental disorders (NDD). Human studies show the deleterious impact of prenatal inflammation and low n-3 polyunsaturated fatty acid (PUFA) intake on neurodevelopment with long-lasting consequences on behavior. However, the mechanisms linking maternal nutritional status to MIA are still unclear, despite their relevance to the etiology of NDD. We demonstrate here that low maternal n-3 PUFA intake worsens MIA-induced early gut dysfunction, including modification of gut microbiota composition and higher local inflammatory reactivity. These deficits correlate with alterations of microglia-neuron crosstalk pathways and have long-lasting effects, both at transcriptional and behavioral levels. This work highlights the perinatal period as a critical time window, especially regarding the role of the gut-brain axis in neurodevelopment, elucidating the link between MIA, poor nutritional habits, and NDD.

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INTRODUCTION

Neurodevelopmental disorders (NDD), such as Autism Spectrum Disorders (ASD), intellectual Disability or Attention Deficit Hyperactivity Disorder (ADHD), are a group of disabilities that arise from a disrupted development of the central nervous system (CNS) [1]. Individuals with NDD display cognitive deficits, associated with hippocampal dysfunction, among other symptoms [1]. Despite the identification of several genetic risk factors, they do not entirely explain NDDs. These disorders can also originate from complex interactions between various environmental factors [2]. Epidemiological studies reveal that maternal immune activation (MIA) or poor nutritional habits in the perinatal period are among the strongest of risk factors for NDD [3–8].

Long term structural and behavioral deficits relevant to NDD are commonly observed in the offspring of rodent models of MIA, including memory deficits and functional alterations in the hippocampus [6, 9–15]. Furthermore, some nutrients, including the polyunsaturated fatty acids (PUFAs), have a profound long-term influence on brain function [5, 16, 17]. PUFAs are essential fatty acids required for proper brain development and maturation [18–25]. Because they must be provided by alimentation, low dietary intake of these nutrients strongly affects neurodevelopment [8]. The principal forms of PUFAs in the CNS are the long chain (LC) arachidonic acid (AA, 20:4n-6) and the docosahexaenoic acid (DHA, 22:6n-3) [16]. Both clinical and preclinical studies show that low levels of LC n-3 PUFAs increase the risk of NDD and/or aggravate symptoms [8, 16, 26]. Recent clinical evidence suggests that n-3 PUFA homeostasis may be altered in ASD, either as a result of nutritional imbalance or genetic defect [27]. Finally, we and others already showed that decreasing n-3 PUFA dietary intake can affect the offspring's susceptibility to MIA [8, 28]. However, the mechanisms linking the early-life nutritional status and inflammation to later life behavioral alterations are still unclear.

Microglia are essential for brain maturation, hence, interfering in their developmental activity gives rise to NDD-like symptoms in mice [9, 29–31]. In normal conditions, microglia guide axons, phagocyte apoptotic neurons, refine spines and synapses, in an activity-dependent manner [29, 30, 32–43]. Both MIA and nutritional imbalance have been shown to disrupt these processes, which might explain the long-term structural and behavioral defects [8, 9, 16, 44]. Myelination is also sensitive to early-life insults and a decrease in white matter integrity is a marker of most, if not all, NDDs [45, 46]. Both MIA and n-3 PUFAs can modulate myelination according to preclinical and clinical studies [47–57].

More recent literature highlights the role of the gut-brain axis in the occurrence of NDD [58–61]. Clinical studies have observed alterations in the composition of microbiota and associated metabolites in subjects with autism and schizophrenia [62–68].

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The gut microbiome plays an important role in the modulation of microglial function and myelination and both PUFAs and MIA can alter its composition [69–74].

In the present study, we show that (1) n-3 PUFA deficiency reveals MIA-induced behavioral alterations in adult offspring but not in pups, (2) both n-3 PUFA deficiency and MIA affect brain lipid composition in adults, (3) this correlates to profound transcriptomic alterations in adulthood, especially for genes involved in CNS development, (4) n-3 PUFA deficiency worsens the impact of MIA on microglia-neuron crosstalk and alters the expression of oligodendrocyte and myelin markers in the developing brain, (5) n-3 PUFA deficiency worsens MIA-induced microbiota and gut alterations in pups, (6) microbial composition and gut inflammatory molecules correlate with neurobiological outcome during the post-natal period.

MATERIAL AND METHODS

Animals

Animal husbandry and experimental procedures were in accordance with the EU Directive 2010/63/EU for animal experiments and approved by the Bioethical committee of our University (no. 50120186-A) and Région Aquitaine Veterinary Services (Direction Départementale de la Protection des Animaux, approval ID: A33-063-920). Every effort was made to minimize suffering and the number of animals used. All experiments were conducted in CD1 mice (Charles River, Arbresle, France). Mice were maintained under standard housing conditions in a temperature (23 ± 1 °C) and humidity (40–50%) controlled animal room with a 12h/12h light/dark cycle (07 h–19 h) and ad libitum access to food and water.

Diet and treatment

α-linolenic acid (ALA, 18:3 n-3) and linoleic acid (LA, 18:2 n-6) are the dietary precursors of omega-3 and omega-6 respectively. As soon as the male was introduced in the mating cage, female mice were fed isocaloric diets containing 5% fat with a high or low LA(n-6)/ALA(n-3) ratio (n-3 deficient group or "DEF" and n-3 sufficient group or "SUFF," respectively) across gestation and lactation (i.e., from the first day of gestation until the post-natal day –PND-21) [28, 75–79]. These diets were custom-made and the pellets were prepared by the SAAJ-INRAE laboratory (Jouy-en-Josas, France).

Females were exposed to males for 48 h. Mating was further confirmed by the observation of a vaginal plug and by measuring weight gain across gestation. At embryonic day 17 (E17), pregnant females were given an intraperitoneal (i.p.) injection of lipopolysaccharide (LPS, E. Coli LPS0127:B8, Sigma Inc, St. Louis, MO, USA; 0.12 µg/g mouse/100 µl). We assessed the development of a sickness behavior in dams by comparing their body weight at E17 and E18 (24 h after the administration of LPS). As expected, we found a significant decrease in body weight in both n-3 sufficient and n-3 deficient mice (Supplementary Fig. 1). We previously showed that this dose of LPS was sufficient to induce an inflammatory response in fetuses' brain, which was exacerbated by n-3 PUFA deficiency [28]. The administration of the corresponding volume of saline solution (NaCl 0.9%, "Saline") was used as a control [28, 80, 81]. Sickness behavior and weight were monitored at E17 and E18 to confirm that dams treated with LPS displayed a significantly lower weight gain than saline-treated dams (see MIA checklist, Table S1; [82]).

At birth, litter size was limited to 10 pups. At PND21, the weaned males were housed in groups of 3–6 without mixing the litters. We generated between 2 and 5 litters per condition. Only 1–2 pups from each litter were tested, to avoid any litter effect.

Cohorts were organised as follows (Fig. 1a): (1) One cohort for neonates' behavior (PND4-8) (Fig. 1); (2) One cohort to assess microglial density/phenotype, microglia-neuron crosstalk and neuronal morphology between PND14 and PND28 (Fig. 3); (3) One cohort dedicated to gut assessment (intestinal permeability, microbiota composition). The brains of these mice were used to conduct immunohistochemistry studies (Iba-1, myelin markers) and perform correlation studies (Figs. 4, 5); (4) 2 cohorts were used for the behavioral assessment of the adult offspring (one for locomotion and anxiety and another for memory testing by the Morris Water Maze) (Fig. 1); (5) one cohort for lipid and transcriptomic profiling in the hippocampus of adults (Fig. 2).

All methodological details appear as supplementary information.

Statistical analysis

All data are expressed as means ± SEM. Normality and homoscedasticity of distributions were assessed by Shapiro-Wilk test and Brown–Forsythe test respectively. Data were then analysed using parametric two-way analysis of variance (ANOVA) (diet and prenatal treatment as factors) followed by Bonferroni post-hoc test when applicable. If normality and/or homoscedasticity tests failed, data were analyzed with a non-parametric Kruskal–Wallis test followed by Mann–Whitney U test when applicable. To analyse recognition index results in the probe test of the Morris Water Maze, we performed a one sample *t* tests (comparison to chance level set at 25%). To calculate the inflammation Z-score, all cytokines were converted to Z-scores (each cytokine value subtracted by the mean of the group and divided by the SD), and the Z-scores were added. Spearman correlations were used to explore associations between microbial composition, the gut immune cells reactivity and markers of myelin and oligodendrocytes. All analyses were conducted with GraphPad Prism 7 (GraphPadSotfware, San Diego, USA) except for repeated measures analysis (learning and reversal phase in Morris Water Maze task; pups' exploratory behavior), where we used Statistica 6.0 (StatSoft, Tulsa, USA) and for correlation matrix where we used R version 3.5.2. For all results, statistical significance was set at $p < 0.05$. Details for all statistical results are presented in Tables S2 and S3.

RESULTS

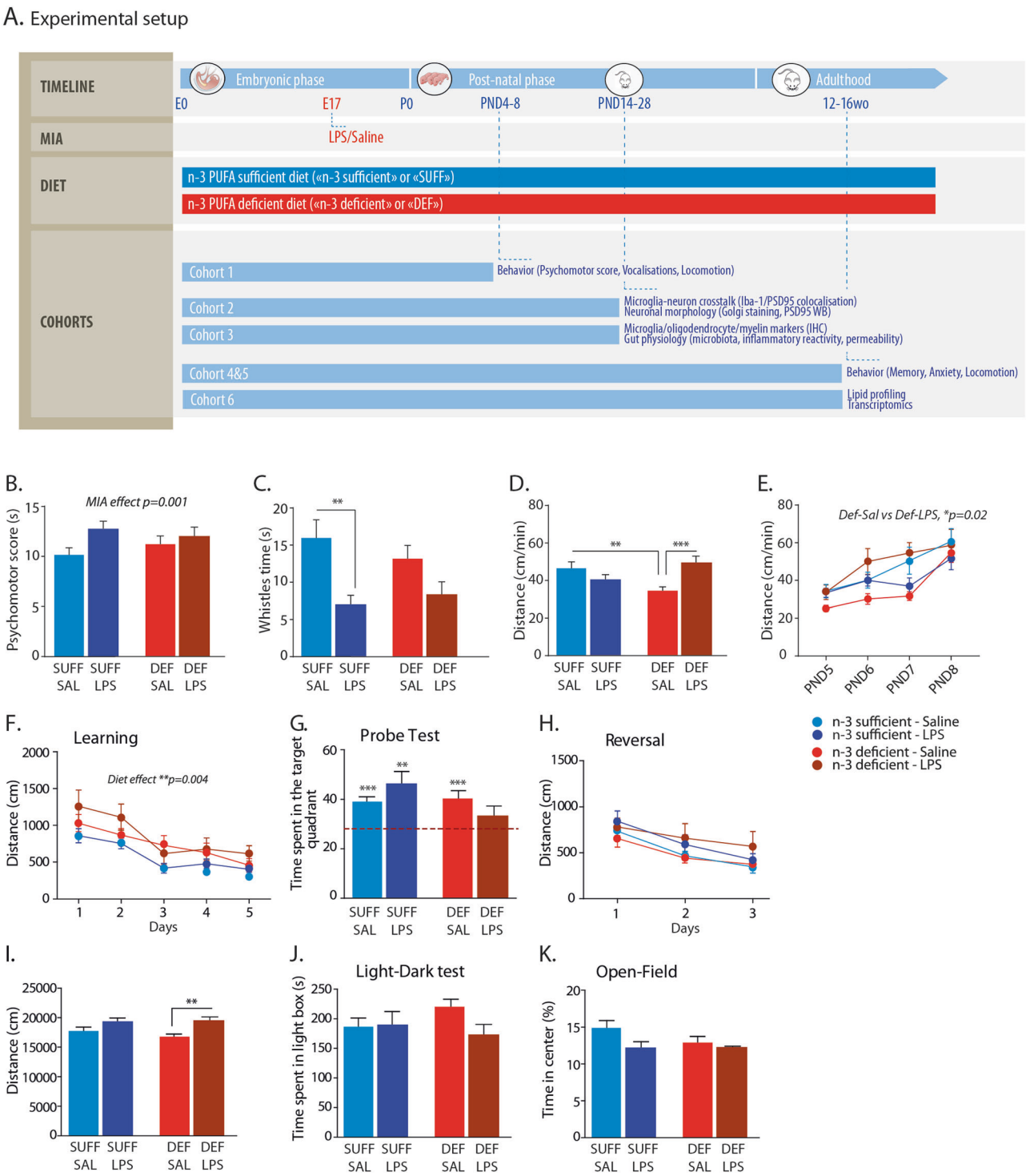
Low maternal n-3 PUFA dietary intake results in MIA-induced locomotor and spatial memory deficits in adult offspring

We assessed short and long-term consequences of MIA and dietary manipulation on offspring's behavior. Regardless of the diet, MIA negatively affected the psychomotor score of pups between PND4 and PND6, measured by the increase of time needed to perform the 2 tests of the Fox battery. In addition, MIA decreased the whistles time of vocalizing PND7-8 pups in response to maternal separation, independent of maternal diet. (Fig. 1b, c). The exploratory behavior of neonates was significantly decreased in n-3 deficient mice (Fig. 1d, e). Moreover, MIA significantly increased the mean exploratory behavior of n-3 deficient animals.

In adulthood, n-3 deficient mice displayed learning deficits during the training phase of the Morris Water Maze task (Fig. 1f). During the probe test, MIA-exposed n-3 deficient mice spent the same amount of time in all 4 quadrants of the maze, a marker of spatial memory deficits (Fig. 1g). When assessing locomotor activity, MIA-exposed n-3 deficient mice traveled significantly longer distances than n-3 deficient-Saline or n-3 sufficient mice during the 1 h-recording period (Fig. 1i).

Finally, we could not find any effect of MIA or diet on the reversal task in the Morris Water Maze, or on the anxiety levels as assessed in the light-dark box and the open-field (data not shown) (Fig. 1h–k).

Overall, our data showed that MIA induced spatial memory deficits and hyper-locomotion only in n-3 deficient adult offspring.



MIA-exposed n-3 deficient adult mice display alterations in the metabolism of n-6 PUFAs in the hippocampus

N-3 PUFA dietary deficiency significantly altered fatty acid composition of the hippocampus in the adult offspring (Fig. 2a–d, Table 1, Table S3), including an increase in the total amount of n-6 PUFAs, Saturated Fatty Acids and MonoUnsaturated Fatty Acids, and a decrease in n-3 PUFAs. MIA alone also increased SFA concentration and decreased total PUFAs, total n-6 PUFAs, DocosaTetraenoic Acid (DTA), and DPA levels (Table 1 and Table S3). Post-hoc analysis revealed that these effects were more pronounced in MIA-exposed n-3 deficient mice (Fig. 2a–d).

Dietary n-3 PUFA deficiency exacerbates MIA-mediated alterations of gene expression in the adult hippocampus

Next, we used transcriptomics to assess gene regulations sustaining the behavioral effects of n-3 PUFA deficiency and MIA in adults. We focused on the hippocampus, a critical structure for learning and memory abilities [83]. When comparing MIA-exposed mice to saline-treated individuals, 337 genes were significantly differentially expressed (DEGs) in n-3 sufficient mice vs 610 DEGs in n-3 deficient animals, among which 120 genes were common to both dietary groups (Tables 2, 3). This suggests that MIA had a greater impact on gene expression under low n-3 PUFA intake

Fig. 1 Effect of n-3 PUFA deficiency on MIA-induced behavioral deficits in neonates and in adult offspring. All graphs show Means ± SEM. **a** Experimental setup. **b** Average time spent by pups to achieve the Fox battery tests (negative geotaxis and righting reflex; 3 trials per day from PND4 to PND6). $N = 14–19$. Two-way ANOVA: MIA effect, $F(1,62) = 11.67$, $p = 0.0011$. **c** Average vocalization time (15-min sessions at PND7-8). $N = 14–19$. Kruskal–Wallis test followed by Mann–Whitney comparison; n-3 sufficient-Saline vs n-3 sufficient-LPS, $^{**}p < 0.01$. **d** Neonate average locomotion measured as the distance traveled (in cm/min) during 1 min-session from PND5 to PND8. $N = 14–19$. Kruskal–Wallis test followed by Mann–Whitney comparison; n-3 sufficient-Saline vs n-3 deficient-saline, $^{**}p = 0.009$, n-3 deficient-Saline vs n-3 deficient-LPS, $^{***}p < 0.001$. **e** Time course of locomotor activity of newborns from PND5 to PND8. $N = 14–19$. Two-way ANOVA on repeated measures followed by Bonferroni's multiple comparisons test: n-3 deficient-Saline vs n-3 deficient-LPS, $^{*}p = 0.02$. **f** Time course of the distance traveled in the Morris Water Maze during the learning phase (in cm). $N = 10$. Two-way ANOVA on repeated measures: diet effect, $F(1,36) = 9.22$, $p = 0.004$. **g** Percentage of time spent in the target quadrant. $N = 10$. One-sample *t* test; n-3 sufficient-Saline, $^{***}p < 0.001$; n-3 sufficient-LPS, $^{**}p = 0.004$; n-3 deficient-saline, $^{***}p < 0.001$; n-3 deficient-LPS, $p = 0.11$. **h**. Time course of the distance traveled in the Morris Water Maze during the reversal learning phase (in cm). $N = 10$. Two-way ANOVA on repeated measures: MIA effect, $F(1,36) = 3.27$, $p = 0.008$; time effect, $F(1,36) = 19$, $p < 0.001$. **i** Basal locomotor activity (in cm). $N = 12$. Kruskal–Wallis test followed by Mann–Whitney comparison; n-3 deficient-Saline vs n-3 deficient-LPS, $^{**}p = 0.008$. **j** Time spent in the light box (anxiogenic area) of the light-dark test. $N = 11$. Kruskal–Wallis test followed by Mann–Whitney comparison. **k** Percentage of time spent in the center of the open-field arena (anxiogenic area). $N = 8–11$. Kruskal–Wallis test followed by Mann–Whitney comparison.

(Fig. 2e). Figure 2f, g illustrates the most significantly dysregulated genes in n-3 sufficient (Fig. 2f) and n-3 deficient mice (Fig. 2g). Principal component analysis (PCA), using DEGs between n-3 sufficient-LPS vs n-3 deficient-LPS mice, showed separation between dietary groups (Fig. 2h).

We then used the MGI database [84] to retrieve the non-redundant Gene-Ontology annotations of interest that are related to the set of DEGs identified above. We computed the number of genes for each selected GO-term, in every group of DEGs identified for each diet type (Fig. 2i, j). The analysis revealed that the majority of these genes belong to "Cell communication" (e.g., synaptic activity, neurotransmitter release), "Immune/defense response" (e.g., leukocyte activation/migration/homeostasis), "CNS development" (e.g., myelination, gliogenesis, neurogenesis) or "Lipid metabolism" (e.g., fatty acid metabolism, membrane lipid metabolic process) pathways (Fig. 2i, j). Based on these transcriptomic data, we further analysed the impact of low n-3 PUFA intake and exposure to MIA on: (1) microglia-neuron crosstalk in the developing hippocampus; (2) neuronal phenotype; (3) myelination.

MIA induces persistent neuronal morphology alterations in n-3 deficient mice, likely by modifying microglia-neuron interactions

During the first post-natal weeks, microglia prune nonfunctional and immature synapses as a maturation mechanism for neuronal networks [29, 30, 85]. We quantified the level of colocalization between microglia (Iba-1) and dendritic spines (PSD95) at PND14, when synaptic refinement peaks in the hippocampus [86]. Our data show that n-3 PUFA deficiency significantly increased the level of Iba-1/PSD95 colocalization at PND14, suggesting greater synaptic pruning activity (Fig. 3a). Prenatal exposure to LPS did not show any effect. However, MIA differentially affected the expression of *c3*, *cd47*, *cx3cr1*, *cx3cl1* according to n-3 PUFA intake, all these genes being involved in microglia-mediated synaptic pruning in the developing brain (Fig. 3b). These effects could not be attributed to modifications of microglial density (quantification of Iba-1 immunoreactivity) and/or phenotype (flow cytometry analysis of phenotypic markers on sorted microglia) (Supplementary Fig. 2a, b). We could only observe a slight, yet significant, increase in the proportion of CD86+ microglia in MIA-exposed n-3 deficient mice at PND14, suggesting that the cells may be skewed towards a proinflammatory phenotype (Supplementary Fig. 2b) [87]. Prenatal LPS exposure also did not affect cytokine mRNA production in the hippocampus of the offspring (Supplementary Fig. 2c).

We further studied the consequences of altered microglia-neuron interactions on neuronal morphology at PND28, when most of the synaptic refinement is complete in the hippocampus. N-3 PUFA deficiency significantly decreased spine density (Fig. 3c). We also observed that MIA-exposed n-3 deficient mice had greater number of spines on pyramidal neuron and expressed higher amount of PSD95 protein vs n-3 deficient-Saline animals (Fig. 3c, d). MAP2 protein expression was similar across all experimental groups (Supplementary Fig. 2d).

Overall, our data suggest that n-3 PUFA deficiency affected the amplitude of response to MIA in terms of dendritic spine density and microglia-neuron communication during the post-natal period.

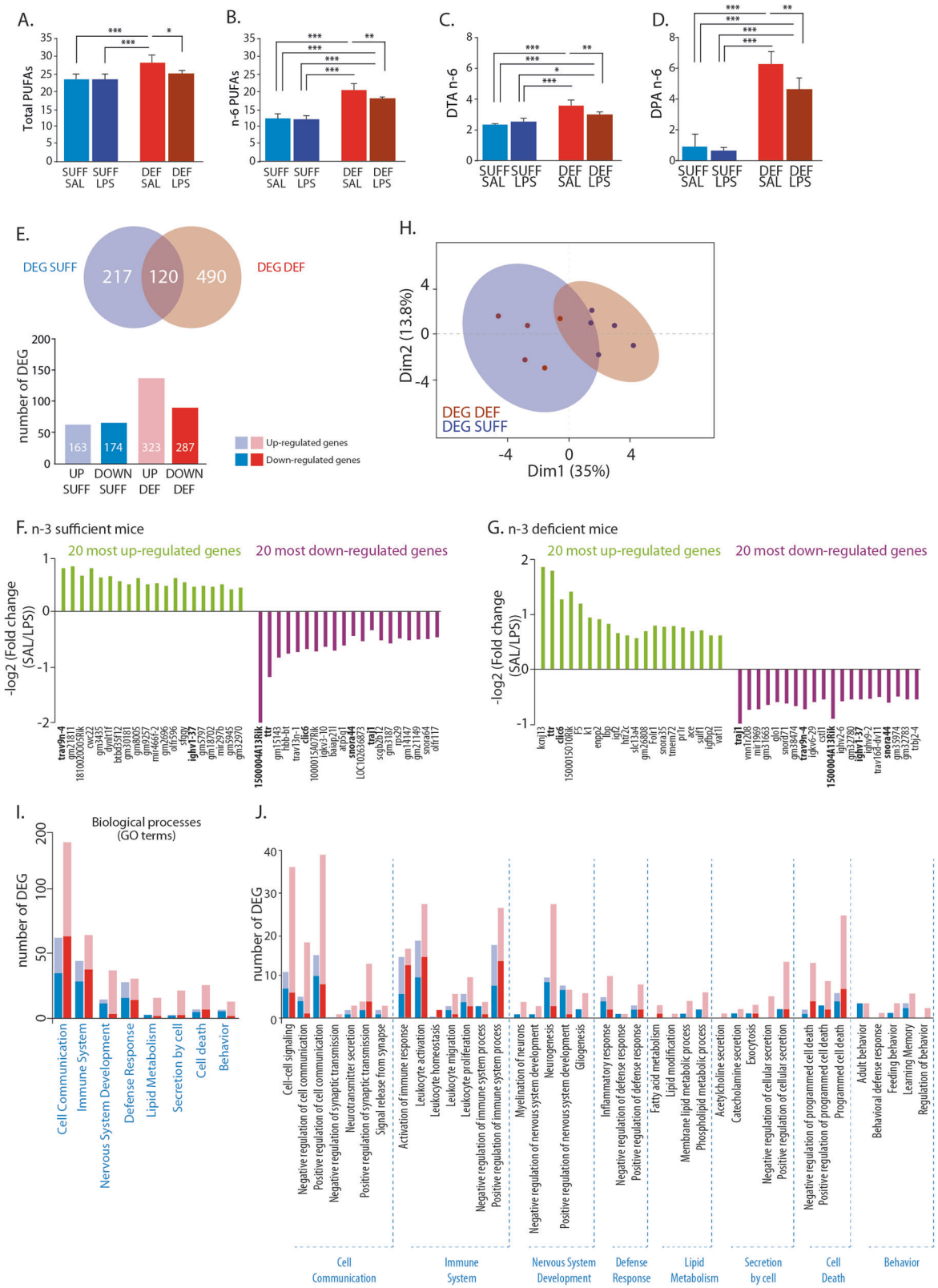
No interaction between MIA and n-3 PUFA deficiency on myelin and oligodendrocyte protein expression

According to the transcriptomic analysis, "gliogenesis" and "myelination" processes are likely to be altered by MIA and n-3 PUFA deficiency (Fig. 2j). MIA significantly reduced the expression of Olig2 (marker of all oligodendrocytes) and PLP (mature oligodendrocytes marker) at PND14, the peak of oligodendrocyte maturation and myelination in the developing brain (Fig. 3e, f). APC protein (for Adenomatous Polyposis Coli, a marker for mature oligodendrocytes) levels were significantly increased by n-3 PUFA deficiency while the expression of the myelin proteins MAG and MBP were not altered (Fig. 3g–i). Overall, we did not observe any interaction between MIA exposure and low n-3 PUFA dietary intake on oligodendrocyte and myelin protein expression.

MIA differentially alters microbiota composition in both dietary groups and reveals gut alterations under n-3 PUFA deficiency

A growing body of evidence suggests microbial modulation of brain functions, especially when occurring during the post-natal developmental phase [12, 59, 61, 69, 70, 74, 88–107]. We therefore assessed the gut microbiota at PND14 and PND21, i.e., during a critical time window for gut physiology and gut-brain communication. Alpha diversity was significantly higher in n-3 sufficient-Saline group than in any other group, at both time points, (Fig. 4a–e; Tables 4, 5). While n-3 PUFA deficiency decreased diversity at both time points, at PND21 MIA restored microbial diversity up to control levels in n-3 deficient mice (Fig. 4e; Table 5). A strong effect of diet and MIA was observed at phyla, family and genus levels (Fig. 4b, c–f, g; Tables 4, 5). At both ages, n-3 PUFA deficiency increased the amount of Proteobacteria and decreased the proportion of Lachnospiraceae. It also decreased the amount of Prevotellaceae and increased Akkermansiacaceae and Enterobacteriaceae at PND14, and decreased the Lactobacillaceae and increased the Tannerellaceae and Rikenellaceae at PND21 (Fig. 4c–g). All groups clustered together by PCA analysis at PND14, while they clustered distinctly from controls (n-3 sufficient-Saline) at PND21 (Fig. 4d–h). Our data revealed a robust effect of MIA and low n-3 PUFA intake, alone and combined, on gut microbiota composition, in an age-dependent manner.

At PND14, n-3 PUFA deficiency significantly shortened colon length and increased its permeability. MIA displayed no effect on these parameters (Supplementary Fig. 3a, b). At PND21, n-3 deficient-Saline mice still exhibited shorter colon but lower



permeability compared with n-3 sufficient group. MIA increased both parameters in n-3 deficient mice while it had no effect in mice fed n-3 PUFA sufficient diet (Supplementary Fig. 3c, d).

We then assessed mesenteric lymph node T-cells cytokine secretion as a marker of gut immune reactivity [59, 108]. T cells sorted from PND14 n-3 deficient-LPS mice released greater levels of TNFα, IFN-γ, and IL-17 upon anti-CD3/anti-CD28 stimulation compared with n-3 sufficient-LPS group, while production of the anti-inflammatory cytokine IL-10 was hampered (Fig. 4i). We calculated a z-score that summarizes the cytokine profile of mice

Fig. 2 Dietary n-3 PUFA deficiency exacerbates MIA-induced alterations of the hippocampal lipid and transcriptional profiles in adulthood. Quantification of the levels of total PUFAs (**a**), n-6 PUFAs (**b**), DTA n-6 (**c**) or DPAn-6 (**d**) in the hippocampus of adult mice, expressed as the percentage of total fatty acids. All graphs show Means ± SEM. $N = 6$. Two-way ANOVA followed by Bonferroni's multiple comparisons test: Total PUFAs: n-3 sufficient-Saline vs n-3 deficient-Saline, ***$p = 0.0001$; n-3 deficient-Saline vs n-3 deficient-LPS, *$p = 0.0156$; n-3 deficient-Saline vs n-3 sufficient-LPS, ***$p = 0.0003$. Total n-6 PUFAs: n-3 sufficient-Saline vs n-3 deficient-Saline, ***$p < 0.0001$; n-3 deficient-Saline vs n-3 deficient-LPS, **$p = 0.0055$; n-3 deficient-Saline vs n-3 sufficient-LPS, ***$p < 0.0001$; n-3 sufficient-Saline vs n-3 deficient LPS, ***$p < 0.0001$; n-3 deficient-LPS vs n-3 sufficient-LPS, ***$p < 0.0001$. DTA *n-6*: n-3 sufficient-Saline vs n-3 deficient-Saline, ***$p < 0.0001$; n-3 deficient-Saline vs n-3 deficient-LPS, **$p = 0.0013$; n-3 deficient-Saline vs n-3 sufficient-LPS, ***$p < 0.0001$; n-3 sufficient-Saline vs n-3 deficient LPS, ***$p = 0.0004$; n-3 deficient-LPS vs n-3 sufficient-LPS, *$p = 0.0158$. *DPA n-6*: n-3 sufficient-Saline vs n-3 deficient-Saline, ***$p < 0.0001$; n-3 deficient-Saline vs n-3 deficient-LPS, **$p = 0.0045$; n-3 deficient-Saline vs n-3 sufficient-LPS, ***$p < 0.0001$; n-3 sufficient-Saline vs n-3 deficient LPS, ***$p < 0.0001$; n-3 deficient-LPS vs n-3 sufficient-LPS, ***$p < 0.0001$. **e** Venn diagram highlighting the number of genes that were modulated by MIA in the hippocampi of adult n-3 sufficient (blue) or n-3 deficient (red) mice. Lower panel: Number of genes that were up- or down-regulated in n-3 sufficient and n-3 deficient mice. Representation of the 20 most significantly dysregulated genes in n-3 sufficient (**f**) and n-3 deficient (**g**) mice. Genes that appear in both n-3 sufficient and n-3 deficient mice are bold. **h** PCA analysis of MIA-induced differentially expressed genes (DEG) in both dietary groups. Confidence ellipses appear around each group. **i**, **j** Gene Ontology analysis of DEGs (light red and blue: up-regulated genes; dark red and blue: down-regulated genes).

Table 1. Total fatty acid composition of adult hippocampus.

	n-3 balanced		n-3 deficient		Statistical effects		
Fatty acids	Saline	LPS	Saline	LPS	LPS	Diet	LP × Diet
SFA	44.3 ± 1.89	45.0 ± 1.98	41.2 ± 1.81	44.4 ± 1.31	<0.05	<0.05	NS
MUFA	23.3 ± 1.40	22.7 ± 0.72	21.2 ± 2.03	21.2 ± 0.86	NS	<0.01	NS
PUFA	23.3 ± 1.58[b]	23.4 ± 1.33[b]	28.2 ± 2.09[a]	25.2 ± 0.72[b]	<0.05	<0.001	<0.05
DMA	9.1 ± 0.64	8.9 ± 1.18	9.4 ± 0.78	9.2 ± 0.44	NS	NS	NS
18:2 n-6 (LA)	0.47 ± 0.24	0.37 ± 0.13	0.44 ± 0.03	0.49 ± 0.06	NS	NS	NS
20:3 n-6 (DGLA)	0.34 ± 0.03	0.35 ± 0.04	0.26 ± 0.04	0.27 ± 0.05	NS	<0.001	NS
20:4 n-6 (AA)	8.16 ± 0.77	8.08 ± 0.55	9.74 ± 1.07	9.39 ± 0.19	NS	<0.001	NS
22:4 n-6 (DTA)	2.34 ± 0.08[c]	2.54 ± 0.20[c]	3.58 ± 0.34[a]	3.00 ± 0.18[b]	<0.05	<0.001	<0.001
22:5 n-6 (DPA)	0.88 ± 0.85[c]	0.65 ± 0.15[c]	6.29 ± 0.80[a]	4.63 ± 0.76[b]	<0.01	<0.001	<0.05
n-6 PUFAs	12.2 ± 0.99[c]	12.0 ± 0.89[c]	20.3 ± 1.73[a]	17.8 ± 0.40[b]	<0.01	<0.001	<0.05
18:3 n-3 (ALA)	0.40 ± 0.11	0.40 ± 0.06	0.37 ± 0.10	0.34 ± 0.04	NS	NS	NS
22:5 n-3 (DPA)	0.16 ± 0.07	0.13 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	NS	<0.01	NS
22:6 n-3 (DHA)	10.2 ± 0.88	10.6 ± 0.66	7.31 ± 0.90	6.83 ± 0.69	NS	<0.001	NS
Total n-3	10.8 ± 0.80	11.2 ± 0.67	7.77 ± 0.85	7.25 ± 0.71	NS	<0.001	NS
n-6/n-3	1.13 ± 0.06	1.08 ± 0.07	2.64 ± 0.31	2.47 ± 0.26	NS	<0.001	NS

$n = 5$–6 in all groups. Values with different superscripts ([a,b,c]) differ significantly ($p < 0.05$).
SFA saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *LA* linoleic acid, *DGLA* di-homo-gamma-linolenic acid, *AA* arachidonic acid, *DTA* docosatetraenoic acid, *DPA* docosapentaenoic acid, *ALA* α-linolenic acid, *DHA* docosahexaenoic acid.

and confirmed that the effect of MIA on cytokine release was more pronounced in n-3 deficient mice (Fig. 4k). At PND21, MIA increased cytokine production more drastically in stimulated T cells sorted from n-3 sufficient mice (Fig. 4j, l). At histological levels, inflammation (Ameho score) was not different between groups (Supplementary Fig. 3e, f).

Hence, n-3 PUFA deficiency enhanced gut immune reactivity during the post-natal period, shifting the MIA-mediated proinflammatory response towards the earlier time point.

Microbial modifications correlate with gut inflammatory reactivity and neurobiological alterations

Spearman's correlation matrix between gut immune cells reactivity and bacterial genera show that the microbial composition was tightly correlated with gut inflammatory reactivity both at PND14 and PND21 (Fig. 5a–c). Our data also revealed correlations between microbial composition and markers of myelin (olig2, MAP2, PLP) and microglial cells (Iba-1) (Fig. 5b–d).

We further assessed potential correlations between gut inflammatory reactivity and neurobiological features at PND21. Spearman correlation analysis revealed a strong correlation between Iba-1 and T cells-induced cytokines (Fig. 5e). We also observed significant correlation between Olig2 expression and the production of TNF-α and IL-17 (Fig. 5e). This suggests that alterations of gut physiology are linked to neurobiological defects in the developing brain.

DISCUSSION

We and others previously showed that maternal dietary n-3 PUFA intake influences neurobiological and behavioral outcome in mice exposed to prenatal inflammation [6, 9, 28, 109, 110]. Our study is the first to extensively explore the mechanisms underlying the cumulative effects of MIA and n-3 PUFA deficiency, along the gut-brain axis and at various ages (Fig. 5f). We show that (1) n-3 PUFA dietary deficiency reveals MIA-induced behavioral alterations in adult offspring but not in pups, (2) MIA-exposed n-3 deficient adult mice display alterations in the metabolism of n-6 PUFAs in the hippocampus. (3) This was correlated to profound transcriptomic alterations, especially for genes involved in CNS development processes, such as synaptic plasticity, myelination and inflammation. (4) N-3 PUFA deficiency worsened the impact of MIA on microglia-neuron interaction in pups. (5) N-3 PUFA deficiency worsened MIA-induced microbiota and gut alterations

Table 2. List of differentially expressed genes (DEGs) in all experimental groups (comparison saline vs LPS in bofth dietary groups).

n-3 sufficient down-regulated genes				n-3 sufficient up-regulated genes				n-3 deficient down-regulated genes				n-3 deficient down-regulated genes			
GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP
1500004A13Rik	4.018	4.41E–13	1.116E–08	Trav9n-4	0.5779	3.65E–09	9.245e-05	Traj1	1.966	4.67E–10	1.181E–05	Kcnj13	0.2752	8.54E–12	2.161e–07
Ttr	2.265	2.24E–09	2.831E–05	Gm21811	0.5643	1.46E–08	0.0001851	Vmn1r208	1.665	1.62E–08	0.0002053	Ttr	0.2894	4.12E–11	5.218e–07
Gm15143	1.781	1.64E–08	0.0001385	1810020O05Rik	0.6358	5.01E–08	0.0004223	Mir1969	1.652	2.28E–08	0.0001923	Clic6	0.4155	1.78E–09	1.503e–05
Hbb-bt	1.687	1.94E–08	0.0001228	Cwc22	0.5791	7.48E–08	0.0004729	Gm31663	1.553	1.33E–07	0.0008417	1500015O10Rik	0.3763	2.21E–09	1.396e–05
Trav13n-1	1.653	5.55E–08	0.0002342	Gm3435	0.6517	9.13E–08	0.000462	Glo1	1.51	2.23E–07	0.001129	F5	0.4356	5.62E–09	2.845e–05
Clic6	1.605	6.87E–08	0.0002483	Dynlt1f	0.6424	2.08E–07	0.0008768	Snord71	1.532	6.15E–07	0.002592	Kl	0.52	1.49E–08	6.264e–05
1500015A07Rik	1.643	1.67E–07	0.0005295	Btbd35f12	0.6803	6.73E–07	0.002431	Gm38474	1.579	6.67E–07	0.002411	Enpp2	0.5346	4.99E–08	0.0001803
Igkv3-10	1.561	7.71E–07	0.002167	Gm30181	0.7062	1.28E–06	0.00404	Trav9n-4	1.646	8.42E–07	0.002663	Lbp	0.5628	5.34E–08	0.0001689
Baiap2l1	1.63	8.71E–07	0.002205	Gm8005	0.6566	1.58E–06	0.004438	Igkv6-29	1.502	8.51E–07	0.002394	Igf2	0.6361	7.18E–08	0.0002019
Atp5g1	1.522	9.12E–07	0.002097	Gm9257	0.7081	1.67E–06	0.004235	Cstl1	1.461	8.60E–07	0.002176	Htr2c	0.6489	1.33E–07	0.0003355
Snora44	1.35	3.59E–06	0.007564	Mir466f-2	0.6993	2.20E–06	0.005055	1500004A13Rik	1.849	1.58E–06	0.003635	Slc13a4	0.6732	1.44E–07	0.0003315
LOC102636873	1.447	3.81E–06	0.007419	Gm2696	0.724	3.94E–06	0.008311	Ighv2-6	1.492	1.64E–06	0.003449	Gm26808	0.6175	1.82E–07	0.0003832
Traj1	1.263	4.93E–06	0.008917	Olfr596	0.6533	4.32E–06	0.008415	Gm32780	1.461	2.01E–06	0.003917	Folr1	0.5761	2.14E–07	0.0004159
Scgb2b12	1.429	5.32E–06	0.008978	Fggy	0.6902	6.20E–06	0.01121	Ighv1-37	1.461	2.23E–06	0.004022	Snora35	0.5811	2.73E–07	0.000493
Gm3187	1.489	5.50E–06	0.008697	Ighv1-37	0.7316	6.36E–06	0.01073	Ighv9-2	1.453	2.65E–06	0.004462	Tmem72	0.5807	3.16E–07	0.0005333
Rps29	1.399	5.89E–06	0.008769	Gm5797	0.7223	6.61E–06	0.01046	Trav16d-dv11	1.41	3.53E–06	0.005575	Prlr	0.5892	3.4E–07	0.0005382
Gm14147	1.433	6.15E–06	0.008652	Gm8702	0.73	6.66E–06	0.009913	Snora44	1.518	3.75E–06	0.00558	Ace	0.6158	3.41E–07	0.000508
Gm21149	1.424	1.04E–05	0.01391	Mir297b	0.7057	9.02E–06	0.01268	Gm35974	1.404	3.77E–06	0.005297	Sulf1	0.6134	4.6E–07	0.0006464
Snora64	1.408	1.08E–05	0.01364	Gm5945	0.7529	1.23E–05	0.01638	Gm32783	1.451	3.83E–06	0.005097	Igfbp2	0.65	5.59E–07	0.0007445
Olfr117	1.382	1.23E–05	0.01477	Gm32970	0.7394	1.52E–05	0.01927	Trbj2-4	1.461	4.06E–06	0.005132	Vat1l	0.6486	5.75E–07	0.0007274
Snord45b	1.258	1.44E–05	0.0166	LOC102638110	0.7269	1.57E–05	0.01891	Gm3239	1.411	4.83E–06	0.005822	Prkcd	0.6499	5.91E–07	0.0007124
Traj8	1.391	1.53E–05	0.01685	Olfr153	0.7354	1.61E–05	0.01857	Masp2	1.335	9.04E–06	0.01039	Dynlt1f	0.641	6.33E–07	0.0007281
Slitrk6	1.383	1.66E–05	0.01747	Gm11546	0.753	1.62E–05	0.01786	4930470H14Rik	1.393	9.05E–06	0.009956	Snord14d	0.6741	6.45E–07	0.0007099
F5	1.262	2.01E–05	0.02034	Gm14295	0.7337	1.71E–05	0.01807	Olfr1411	1.381	1.52E–05	0.01599	Rgs16	0.677	9.86E–07	0.00104
Traj39	1.382	2.10E–05	0.02042	Gm14428	0.7537	1.93E–05	0.01957	1700014D04Rik	1.361	1.62E–05	0.01636	Snord14c	0.6835	1.41E–06	0.001424
Gm32112	1.366	2.29E–05	0.02145	Gm15107	0.7534	2.22E–05	0.02156	Gm15091	1.409	1.75E–05	0.01705	Bcl2a1b	0.6758	1.65E–06	0.001607
Vmn1r214	1.351	2.56E–05	0.02309	Trav13n-3	0.7528	2.71E–05	0.02544	Snora17	1.377	1.78E–05	0.01665	Plekhg1	0.6897	1.99E–06	0.001861
1500015O10Rik	1.319	2.74E–05	0.02388	Speer4cos	0.7576	2.74E–05	0.02479	Vmn1r115	1.369	2.24E–05	0.02027	Cldn2	0.6623	1.99E–06	0.001797
Gm35498	1.33	2.75E–05	0.0232	Gm4064	0.7591	3.00E–05	0.02614	Ighv14-2	1.347	2.42E–05	0.0211	Car12	0.6868	2.03E–06	0.00177
Gm3409	1.364	2.75E–05	0.02246	Zfp984	0.7795	3.25E–05	0.0274	Rnu7	1.374	2.44E–05	0.02061	Gm5797	0.6788	2.23E–06	0.001884
Zic1	1.304	3.20E–05	0.02528	Traj21	0.7587	3.26E–05	0.02658	Prn	1.334	2.68E–05	0.02188	Zic1	0.689	2.29E–06	0.001867
Speer4e	1.335	3.25E–05	0.02493	Masp2	0.7888	3.41E–05	0.02697	Mup17	1.367	2.71E–05	0.02139	Mir1912	0.6736	2.41E–06	0.001906
Gm35974	1.321	3.37E–05	0.02508	Mir7679	0.7708	4.07E–05	0.03122	Ccl28	1.366	2.78E–05	0.02135	9330159M07Rik	0.7068	2.59E–06	0.001988
1810037I17Rik	1.358	3.49E–05	0.02522	Gm5169	0.7642	4.48E–05	0.03332	Scgb2b12	1.376	3.51E–05	0.02612	Trav13-4-dv7	0.6736	2.94E–06	0.002185
Traj14	1.39	3.63E–05	0.02548	Gm21720	0.8026	5.12E–05	0.03704	Traj14	1.317	3.57E–05	0.02581	Gpx8	0.7038	2.98E–06	0.002155
Rhox3g	1.354	4.06E–05	0.02777	Olfr205	0.7787	6.61E–05	0.04648	Hist1h2bn	1.375	3.67E–05	0.02577	Tgtp1	0.7007	3.37E–06	0.002366
Olfr628	1.34	4.67E–05	0.03113	Hist1h2bf	0.7779	7.65E–05	0.05235	Olfr24	1.359	3.68E–05	0.02517	Vgll3	0.6972	3.82E–06	0.002609
Invs	1.348	5.48E–05	0.03556	Gm15151	0.7866	8.29E–05	0.0552	4930588K23Rik	1.359	3.78E–05	0.02518	Slc4a5	0.6713	4.71E–06	0.003136
Enpp2	1.215	5.84E–05	0.03692	Luzp4	0.785	9.08E–05	0.05892	Taar5	1.36	4.16E–05	0.02701	Sostdc1	0.6827	5.12E–06	0.003322
H2afb3	1.26	6.71E–05	0.04142	Zfp97	0.7884	9.62E–05	0.06087	Rpl9	1.349	4.30E–05	0.02719	Drc7	0.6859	6.19E–06	0.003913
LOC102637192	1.325	7.21E–05	0.04346	Vmn2r126	0.8594	9.77E–05	0.06027	Tmem181c-ps	1.346	4.42E–05	0.0273	Col8a1	0.7029	9.15E–06	0.005649
Trbj1-5	1.371	7.60E–05	0.04475	Mageb5	0.928	1.00E–04	0.06024	Mir7050	1.336	4.75E–05	0.02859	Nnat	0.735	1.02E–05	0.006168
Vmn2r38	1.326	7.92E–05	0.04554	Tshz2	0.7874	0.0001012	0.05956	Gm36079	1.333	5.04E–05	0.02967	Slc31a1	0.7221	1.18E–05	0.006928
Lbp	1.226	9.76E–05	0.05489	Ighv1-63	0.8207	0.0001077	0.06197	LOC100502592	1.329	5.49E–05	0.03159	Gm30500	0.7348	1.27E–05	0.007311
Kl	1.242	9.94E–05	0.0547	6820431F20Rik	0.8018	0.0001136	0.06388	Gm31255	1.17	5.70E–05	0.03202	Zfp33b	0.7937	1.31E–05	0.007369
Igkv4-62	1.185	0.0001123	0.06046	Olfr406	0.7954	0.0001174	0.06457	Mir423	1.339	5.85E–05	0.03219	Mageb5	0.8057	1.52E–05	0.008356
Folr1	1.141	0.0001299	0.0685	Mir101c	0.7812	0.0001183	0.06371	Trbj1-5	1.327	5.92E–05	0.03186	Syt9	0.7466	1.59E–05	0.008576
Vmn1r119	1.299	0.0001335	0.06895	LOC105246638	0.7807	0.0001237	0.0652	Gm21115	1.338	6.41E–05	0.03382	Trav4-4-dv10	0.7168	1.76E–05	0.009301

Table 2. continued

n-3 sufficient down-regulated genes				n-3 sufficient up-regulated genes				n-3 deficient down-regulated genes				n-3 deficient down-regulated genes			
GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP
4921532D01Rik	1.301	0.0001355	0.06857	Gm6367	0.8347	0.0001248	0.06446	Igkv4-72	1.329	6.93E–05	0.0358	Tmsb15b1	0.7415	1.8E–05	0.009313
Snora35	1.246	0.0001383	0.06863	Gm10406	0.784	0.0001252	0.06336	Trav13n-1	1.203	7.21E–05	0.03649	1810020O05Rik	0.7426	1.93E–05	0.009786
Ulk4	1.285	0.0001588	0.07727	Cox7b	0.7731	0.0001332	0.06611	Mir3968	1.318	7.49E–05	0.03718	Calml4	0.7475	2.01E–05	0.009983
Gm14393	1.213	0.000159	0.0759	Vmn1r52	0.7855	0.0001369	0.06661	Ighv1-78	1.345	7.52E–05	0.0366	Gm13249	0.7506	2.05E–05	0.009973
WITHDRAWN BY NCBI (LOC102633225)	1.301	0.0001636	0.07666	AV320801	0.8241	0.0001375	0.06564	Gm15143	1.205	7.57E–05	0.03615	Mir692-1	0.7577	2.07E–05	0.009865
Ighv8-9	1.289	0.0001656	0.07621	Rxfp1	0.7945	0.0001442	0.06759	Olfr724	1.329	9.13E–05	0.0428	Atp5g1	0.7078	2.1E–05	0.009859
Gm21719	1.297	0.0001663	0.07514	Mir5620	0.8104	0.0001498	0.06893	Olfr224	1.318	9.25E–05	0.04255	Gm13034	0.7503	2.18E–05	0.01003
Sulf1	1.202	0.00017	0.07549	4930503H13Rik	0.7966	0.0001617	0.07309	Scarna2	1.329	0.0001005	0.04542	Rbp1	0.7404	2.29E–05	0.01035
Trpc6	1.294	0.0001948	0.08498	Mir7236	0.7966	0.0001703	0.0756	Traj22	1.341	0.0001061	0.0471	Vmn2r34	0.7635	2.36E–05	0.01048
Tmem72	1.151	0.0001972	0.08457	Bcl2a1b	0.7952	0.0001743	0.07602	Gm2182	1.324	0.0001117	0.04874	Snora30	0.7415	2.44E–05	0.01065
Mir1912	1.117	0.0002065	0.08708	Mup22	0.8371	0.0001809	0.07759	Olfr45	1.306	0.0001261	0.05407	Cwc22	0.6969	2.67E–05	0.01146
Igkv4-79	1.257	0.0002081	0.08632	Vwc2l	0.7943	0.0001822	0.07683	Mir6986	1.298	0.0001436	0.06056	Grp	0.7402	2.81E–05	0.01184
Prkcd	1.295	0.0002089	0.08525	Trav13d-4	0.7952	0.0002229	0.09246	Mptx1	1.312	0.0001477	0.06129	Mrpl20	0.7558	3E–05	0.01246
Olfr926	1.294	0.0002101	0.08437	Vmn2r-ps60	0.819	0.0002268	0.09258	Olfr222	1.31	0.0001488	0.06072	Dcn	0.7362	3.04E–05	0.01239
Snora47	1.29	0.0002126	0.08407	Gm31532	0.8492	0.0002296	0.09221	Snord45b	1.325	0.0001493	0.05998	Postn	0.7355	3.64E–05	0.0146
Akr1e1	1.315	0.0002334	0.09088	Mir421	0.8016	0.0002303	0.09106	Gm1976	1.311	0.0001529	0.06044	4632415L05Rik	0.7526	3.65E–05	0.01445
Gm21825	1.293	0.0002443	0.09366	Trav13-4-dv7	0.8932	0.0002478	0.09646	Gm16427	1.303	0.0001532	0.05965	Gm31981	0.7674	4.34E–05	0.01688
Gm15097	1.29	0.0002501	0.09446	Gapdh-ps15	0.8087	0.0002504	0.096	Igkv4-79	1.297	0.0001535	0.05887	Gm3409	0.762	4.65E–05	0.01781
Calml4	1.232	0.0002596	0.09661	Mir377	0.8083	0.0002537	0.09582	Gm2011	1.289	0.0001589	0.06	Tgtp2	0.7554	4.66E–05	0.0176
Mir7045	1.271	0.000298	0.1093	Snord19	0.7906	0.0002556	0.09512	Gm29720	1.252	0.0001598	0.05946	Gm10087	0.7497	5.57E–05	0.02071
Mup20	1.221	0.0003126	0.113	Igkv4-72	0.8128	0.0002612	0.0958	Ighv1-20	1.304	0.0001748	0.06412	Steap1	0.7571	6.25E–05	0.02291
Ighv1-84	1.292	0.0003237	0.1154	Gsr	0.7989	0.0002652	0.09588	Olfr1532-ps1	1.295	0.0001864	0.06738	Ascc2	0.7773	6.47E–05	0.02338
Gm38474	1.144	0.000325	0.1142	Olfr1347	0.8036	0.000284	0.1012	Ighv5-15	1.29	0.0001927	0.06867	Elovl7	0.7693	6.75E–05	0.02407
Olfr584	1.277	0.0003266	0.1132	Mctp2	0.7976	0.0002865	0.1007	Gm32970	1.27	0.0002162	0.07599	Cfap43	0.7718	7.08E–05	0.02488
Ighv9-2	1.213	0.0003371	0.1153	Trav8-1	0.9279	0.0002975	0.1031	Mir101c	1.259	0.0002188	0.07584	Hist1h2bj	0.7771	7.39E–05	0.02562
Mir6969	1.268	0.0003482	0.1175	Mir297a-4	0.8195	0.0002989	0.1022	Gm5073	1.264	0.0002191	0.07494	Cpne2	0.7668	7.51E–05	0.02569
Ogn	1.145	0.0003539	0.1178	Mir297a-3	0.8158	0.0003053	0.103	Vmn1r177	1.281	0.0002275	0.07675	Olfr153	0.7634	7.72E–05	0.02603
Gm21088	1.301	0.0003541	0.1164	Mir369	0.8093	0.0003138	0.1045	Olfr205	1.302	0.0002293	0.07634	Gm30181	0.7867	7.83E–05	0.02609
Sostdc1	1.187	0.0003585	0.1163	Cntn3	0.8093	0.0003464	0.1138	Vmn2r44	1.266	0.0002313	0.07603	Trpm3	0.7622	7.95E–05	0.02612
Gm30698	1.263	0.0003744	0.1199	Olfr94	0.7977	0.0003502	0.1136	Ighv1-30	1.286	0.000233	0.07559	Snord61	0.763	8.79E–05	0.0285
Gm32125	1.268	0.0003747	0.1185	Gm36806	0.8408	0.0003821	0.1224	LOC102636873	1.279	0.0002373	0.076	Snord65	0.7775	9.31E–05	0.02981
Snord71	1.197	0.0003915	0.1223	Ighm	0.8428	0.0004365	0.1381	Gm3376	1.282	0.0002402	0.07596	Slc2a12	0.7641	9.49E–05	0.03002
Gm16427	1.264	0.0004021	0.1241	H3f3c	0.7996	0.0004369	0.1365	Jmjd7	1.184	0.0002551	0.07971	Rarres1	0.7866	9.76E–05	0.0305
Vmn1r192	1.259	0.0004185	0.1276	Gm5552	0.8133	0.0004471	0.138	Gm21136	1.298	0.0002653	0.08189	Snora20	0.8816	0.0001	0.03087
Gm8579	1.244	0.00042	0.1265	WITHDRAWN BY NCBI (LOC102632416)	0.8725	0.0004532	0.1382	Olfr1170	1.289	0.0002721	0.08295	Pon3	0.7707	0.0001048	0.03195
Igkv4-74	1.263	0.0004462	0.1328	Gm3973	0.8923	0.0004555	0.1372	Gm34905	1.234	0.0002727	0.08215	Atp6v0c-ps2	0.781	0.0001061	0.03198
Ighv1-20	1.268	0.0004682	0.1378	Snord90	0.8507	0.0004563	0.1358	Gm14147	1.286	0.0002785	0.08291	Mir130a	0.7793	0.0001092	0.0325
Mir7662	1.258	0.000483	0.1405	WITHDRAWN BY NCBI (LOC105245735)	0.8169	0.0004581	0.1348	Olfr714	1.276	0.0002981	0.08773	Psmb5	0.7826	0.0001104	0.03249
Mir8094	1.219	0.0004926	0.1416	Gm10087	0.8705	0.0004593	0.1336	Vmn1r10	1.253	0.0003027	0.08804	Gm3164	0.769	0.0001118	0.03251
4930401G09Rik	1.194	0.0005005	0.1423	Gm8050	0.8545	0.0004836	0.1391	9330117O12Rik	1.242	0.0003272	0.09408	Slitrk6	0.7785	0.0001118	0.03214
Ace	1.146	0.0005217	0.1467	Gm31663	0.8133	0.0004889	0.139	Traj50	1.276	0.000346	0.09837	Prr32	0.7811	0.0001119	0.03182
Prpmp5	1.261	0.0005264	0.1464	1700084M14Rik	0.8349	0.0004922	0.1384	Gm3159	1.297	0.000349	0.09812	Btbd35f13	0.7702	0.0001146	0.03223
Mir7040	1.262	0.0005387	0.1482	Ighv8-8	0.8151	0.0005012	0.1394	Traj39	1.221	0.0003506	0.09748	Ucp2	0.7878	0.0001169	0.0325
Btbd35f21	1.281	0.0005543	0.1508	B230217O12Rik	0.8172	0.0005132	0.1412	Snord88a	1.291	0.0003514	0.09666	Gm12666	0.7698	0.000121	0.03327
Prl2c3	1.265	0.0005556	0.1496	Snord64	0.8353	0.0005246	0.1427	Scarna13	1.334	0.0003524	0.09588	Gm3435	0.7737	0.0001294	0.03522
1700091H14Rik	1.255	0.0005612	0.1495	Mir344d-3	0.875	0.0005484	0.1476	Vmn1r238	1.243	0.0003663	0.0986	1700007K13Rik	0.8016	0.0001301	0.03503

Table 2. continued

n-3 sufficient down-regulated genes				n-3 sufficient up-regulated genes				n-3 deficient down-regulated genes				n-3 deficient down-regulated genes			
GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP
Defb35	1.253	0.0005945	0.1567	Snord57	0.8145	0.0005515	0.1469	Gm5941	1.232	0.0003665	0.09762	Spata18	0.7838	0.0001314	0.035
Gm20737	1.251	0.0005962	0.1555	Gm14586	0.8232	0.0005663	0.1493	Mir669k	1.164	0.0003721	0.09809	Abca4	0.7776	0.000137	0.03611
Mir7020	1.255	0.0005986	0.1546	Gm38481	0.8499	0.0005678	0.1481	Eapp	1.186	0.0003794	0.09897	Snord1b	0.7757	0.0001379	0.03599
Hist1h4i	1.23	0.0006633	0.1696	Pcdhb6	0.8155	0.000569	0.1469	Mir3074-2	1.262	0.0003821	0.09866	Gm8290	0.7844	0.0001441	0.03721
Vmn1r37	1.221	0.000698	0.1766	Ighv13-2	0.813	0.0005815	0.1486	Gm14379	1.279	0.0003826	0.09779	Snord49b	0.7644	0.000145	0.03706
Hbb-bs	1.246	0.0007116	0.1783	Mndal	0.9531	0.000615	0.1556	Vmn1r216	1.269	0.0003894	0.09853	Tcf7l2	0.7855	0.0001486	0.03761
A530064D06Rik	1.255	0.0007157	0.1776	Gm13272	0.8481	0.0006254	0.1567	Fthl17f	1.273	0.0003898	0.09765	Snord57	0.7784	0.0001549	0.03881
Gm29865	1.246	0.0007905	0.1942	Trbc2	0.8197	0.000626	0.1553	Gm10538	1.28	0.0004077	0.1012	Gm31458	0.7881	0.0001554	0.03855
Syt9	1.267	0.0008258	0.2009	Gm6551	0.8241	0.0006682	0.1642	Olfr1080	1.261	0.0004114	0.1011	Sdhc	0.7924	0.0001677	0.04119
Gm2411	1.234	0.0008493	0.2047	Mir466q	0.8195	0.0006892	0.1677	Ssxb2	1.264	0.0004176	0.1016	Baiap2l1	0.7374	0.0001717	0.04178
Tdpoz3	1.251	0.0008852	0.2113	Ccl21a	0.8525	0.0007159	0.1709	Il4i1	1.271	0.0004328	0.1043	Rdh5	0.7815	0.0001762	0.04247
Mid1	1.185	0.0009077	0.2147	Gm3636	0.8471	0.000722	0.1708	Fggy	1.136	0.0004433	0.1058	Gm38481	0.7782	0.0001783	0.04256
Cpne9	1.25	0.0009122	0.2137	Gm36712	0.8268	0.0007256	0.17	Olfr763	1.249	0.0004471	0.1057	Scn7a	0.796	0.0001841	0.04355
Olfr10	1.246	0.0009126	0.2119	Zfp991	0.8336	0.000747	0.1734	Zfp934	1.245	0.0004846	0.1135	Mir204	0.7616	0.0001846	0.04325
Gm8232	1.243	0.0009142	0.2103	Gm3285	0.8198	0.0007713	0.1774	Ighv1-76	1.283	0.0004923	0.1143	Synpo2	0.7894	0.0002207	0.05123
Gm32234	1.239	0.0009151	0.2086	LOC102634709	0.8237	0.0007714	0.1759	Gm31210	1.262	0.000493	0.1134	Trav14d-3-dv8	0.7937	0.0002411	0.05547
Snord96a	1.23	0.0009192	0.2077	Gm13249	0.825	0.0007846	0.1773	Gm6890	1.27	0.0004987	0.1137	Cfap44	0.7966	0.0002521	0.05748
Rsph1	1.279	0.0009215	0.2064	Insl3	0.8276	0.000786	0.176	Gm13102	1.25	0.0005074	0.1146	C1ql2	0.7927	0.0002754	0.06223
Gm26808	1.097	0.0009413	0.2089	Atp6v0c-ps2	0.8267	0.0008037	0.1784	Mir7239	1.265	0.0005099	0.1142	Mir7686	0.7958	0.0002794	0.06257
4833415N18Rik	1.244	0.0009482	0.2086	Pin4	0.8547	0.0008288	0.1824	Ighv6-5	1.262	0.0005265	0.1169	Fam216b	0.8252	0.0002796	0.06206
Traj4	1.239	0.0009555	0.2084	Gm14409	0.8418	0.0008315	0.1814	Speer4e	1.271	0.0005288	0.1164	Gm5945	0.7916	0.0003067	0.0675
5830417I10Rik	1.19	0.0009794	0.2118	Gm13034	0.8402	0.0008625	0.1865	1700028B04Rik	1.244	0.0005334	0.1164	Cir1	0.806	0.0003091	0.06743
Serpinb9f	1.234	0.0009927	0.2129	Nat8f4	0.8185	0.0008704	0.1867	Ifna15	1.263	0.0005363	0.116	Otx2	0.7979	0.0003195	0.0691
Igkv4-53	1.231	0.001003	0.2132	Gm3139	0.8285	0.0008881	0.1888	Olfr130	1.238	0.0005638	0.1209	Mndal	0.7862	0.0003253	0.06977
Xlr3b	1.098	0.001006	0.2122	Mir669d-2	0.811	0.0009228	0.1946	Traj17	1.24	0.0005651	0.1202	Fabp7	0.8016	0.0003278	0.0697
1700030N03Rik	1.245	0.001015	0.2122	4921539H07Rik	0.8159	0.0009378	0.1961	Xlr3b	1.152	0.0005706	0.1203	Gmnc	0.7999	0.0003311	0.06983
Gal3st2b	1.16	0.001037	0.2151	Rps13	0.8287	0.0009405	0.1951	Olfr457	1.248	0.000573	0.1198	Snord68	0.8045	0.0003312	0.06927
Vmn2r44	1.176	0.001039	0.2137	Snora34	0.8312	0.0009473	0.1949	Alms1-ps2	1.179	0.00058	0.1203	Cab39l	0.7963	0.000333	0.06908
Crym	1.241	0.001046	0.2136	Nat8f7	0.8232	0.0009547	0.1948	Rxfp1	1.235	0.0005994	0.1233	Calb2	0.7914	0.0003448	0.07093
Mir708	1.187	0.001058	0.2143	Gm7429	0.8336	0.0009618	0.1947	Vmn2r55	1.25	0.0006039	0.1232	AV320801	0.8099	0.0003563	0.07272
Gm9159	1.237	0.001066	0.2142	Nts	0.831	0.0009958	0.2	Taar2	1.267	0.0006066	0.1228	Prkcq	0.8023	0.0003632	0.07352
Tas2r107	1.221	0.00107	0.2131	Ifit1	0.8321	0.00101	0.2012	Gm5154	1.255	0.000609	0.1223	Trbj2-6	0.8597	0.0003664	0.07359
Trav15-2-dv6-2	1.233	0.001085	0.2146	Gm15363	0.8352	0.001032	0.2041	Tdpoz3	1.256	0.0006229	0.1241	Cp	0.8266	0.0003758	0.07488
C230034O21Rik	1.195	0.001093	0.2143	Ly6a	0.8347	0.001064	0.2088	WITHDRAWN BY NCBI (LOC102633225)	1.223	0.0006499	0.1285	Slc16a9	0.8117	0.0003792	0.07496
Abhd1	1.234	0.001095	0.2131	Gm14403	0.8342	0.001087	0.2116	A830005F24Rik	1.254	0.0006502	0.1275	Lef1	0.8084	0.0003809	0.07471
Defb5	1.233	0.001103	0.2132	Olfr524	0.825	0.001097	0.2119	Mir1981	1.253	0.0006514	0.1268	Luzp4	0.8844	0.0003845	0.07485
Olfr1251	1.234	0.001133	0.2172	Blnk	0.8263	0.001124	0.2154	H2afb1	1.222	0.0006629	0.1281	Fxyd1	0.8052	0.0003847	0.07431
Kcnj16	1.239	0.001141	0.217	Mir297a-2	0.8642	0.001179	0.2243	Igkv4-61	1.214	0.0006715	0.1287	Btbd35f12	0.8252	0.0003929	0.07533
Gm35079	1.232	0.001179	0.2227	Tmem181b-ps	0.8401	0.001198	0.2263	Mir196b	1.252	0.0006874	0.1308	Zfp963	0.8135	0.0004131	0.0786
Trav6d-5	1.213	0.001186	0.2223	Zfp971	0.8334	0.001199	0.2247	Gm20257	1.232	0.0006885	0.13	Gm9758	0.8511	0.0004209	0.07948
Gm31621	1.234	0.0012	0.2233	Zfp934	0.8291	0.001205	0.2242	Nt5dc3	1.265	0.0006935	0.13	Snora16a	0.8329	0.000446	0.0836
Mettl7a2	1.182	0.001254	0.2316	AA465934	0.9067	0.001206	0.2228	Gm35077	1.251	0.0007051	0.1312	Ighv1-16	0.9126	0.0004597	0.08553
Trav6n-6	1.237	0.001269	0.2327	Gm39561	0.8342	0.00121	0.2218	Olfr1437	1.249	0.0007166	0.1324	Ctxn2	0.8078	0.000487	0.08996
Mir3095	1.231	0.001284	0.2337	Olfr166	0.8703	0.001248	0.2272	Igkv6-20	1.211	0.0007355	0.1349	Sgms2	0.8087	0.0004911	0.09005
1700020N15Rik	1.268	0.001335	0.2414	4930521O11Rik	0.846	0.001374	0.2483	Mir324	1.248	0.0007451	0.1356	Traj26	0.8155	0.0004981	0.09068
Prlr	1.085	0.00134	0.2404	Crygf	0.8402	0.001379	0.2475	Evi2b	1.227	0.0007921	0.1432	Cdk2ap1	0.8387	0.0004989	0.09018
Defa27	1.143	0.001396	0.2487	Gm5779	0.8338	0.00138	0.246	BB287469	1.221	0.0008243	0.1479	Trpv4	0.8129	0.000513	0.09207

Table 2. continued

n-3 sufficient down-regulated genes				n-3 sufficient up-regulated genes				n-3 deficient down-regulated genes				n-3 deficient down-regulated genes			
GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP
5430401F13Rik	1.21	0.001438	0.2544	Zfp33b	1.012	0.001388	0.2456	Fermt1	1.228	0.0008317	0.1472	Pltp	0.8157	0.0005269	0.09389
Rps16	1.185	0.001442	0.2533	Olfr533	0.8576	0.001441	0.2532	Lce1h	1.248	0.0008327	0.1463	Spint2	0.8128	0.0005504	0.0974
Tmem97	1.226	0.001465	0.2556	Gm6821	0.8426	0.001485	0.2591	Gm13084	1.237	0.000838	0.1462	Tmem255a	0.8144	0.0005525	0.09708
Slc4a5	1.084	0.001466	0.254	Fcrls	0.8865	0.001493	0.2588	Trav6d-4	1.228	0.0008419	0.1459	Snora43	0.8194	0.0005743	0.1002
LOC100502592	1.196	0.001474	0.2537	Gm10142	0.8514	0.001498	0.2579	Traj32	1.235	0.0008437	0.1452	AA413626	0.8294	0.0005814	0.1008
Fmod	1.009	0.001477	0.2526	Snord14c	0.8775	0.001537	0.2628	Tlnrd1	1.242	0.0008558	0.1463	Ltc4s	0.8132	0.0005934	0.1022
Olfr328	1.176	0.001482	0.2516	Mir3474	0.8405	0.001544	0.2623	WITHDRAWN BY NCBI (LOC105245735)	1.249	0.0008595	0.146	Mup10	0.8169	0.0006083	0.104
Defb11	1.233	0.001493	0.2518	Gm3164	0.8486	0.001567	0.2643	Igkv4-63	1.25	0.0008604	0.1451	Olfr1316	0.8183	0.0006154	0.1045
Mir669m-1	1.215	0.001506	0.2524	Rpl36a	0.937	0.001584	0.2654	Ly6g5c	1.243	0.000891	0.1493	Snora2b	0.8068	0.0006235	0.1052
Igfbp2	1.067	0.001591	0.2649	Ighv1-77	0.9033	0.001619	0.2695	Olfr1269	1.222	0.0008945	0.1489	Rnu12	0.8425	0.0006336	0.1062
AI506816	1.204	0.001607	0.2658	Qrfpr	0.8365	0.001665	0.2753	Gm34183	1.244	0.0008968	0.1483	Snord92	0.8179	0.0006384	0.1063
Gm38667	1.224	0.001614	0.2652	1700066C05Rik	0.8438	0.001671	0.2746	Olfr1283	1.249	0.0009044	0.1486	Plcb4	0.8175	0.0006413	0.1061
Gm20063	1.133	0.00163	0.2662	Vmn1r124	0.8533	0.00168	0.2743	Dcpp2	1.245	0.0009107	0.1487	LOC102637947	0.857	0.000643	0.1057
Gpx8	1.183	0.001637	0.2656	Adam4	0.8411	0.001718	0.2787	Olfr1505	1.249	0.0009202	0.1493	Car14	0.8136	0.0006439	0.1051
Ccl28	1.173	0.001645	0.2652	Vmn2r86	0.8432	0.001751	0.2822	A230107N01Rik	1.244	0.0009253	0.1491	Resp18	0.8165	0.0006554	0.1063
Tgtp2	1.221	0.001687	0.2702	Xlr5c	0.9561	0.001793	0.2872	Rps6	1.126	0.0009541	0.1528	Frem1	0.8168	0.0006606	0.1065
Vmn1r60	1.228	0.001696	0.2699	Snord68	0.8484	0.001804	0.287	Trim30b	1.239	0.00096	0.1528	Tmprss11a	0.8231	0.0006687	0.1071
Igkv6-29	1.169	0.001713	0.271	Mir668	0.8425	0.001809	0.2861	Mir5134	1.24	0.0009655	0.1527	Ecm2	0.8205	0.0006713	0.1068
Gm32783	1.239	0.001735	0.2726	Gm3667	0.8429	0.001851	0.2909	Usp17ld	1.222	0.000977	0.1536	Aqp1	0.8239	0.0006716	0.1062
Vaultrc5	1.193	0.001738	0.2715	Gm6570	0.8331	0.001895	0.2961	Gapdh-ps15	1.192	0.0009785	0.1528	Serinc2	0.8227	0.0006978	0.1097
Klhdc7a	1.217	0.001753	0.2721	Jmjd7	0.8407	0.001917	0.2976	Olfr1417	1.247	0.0009937	0.1543	Khdc1c	0.8272	0.0007092	0.1108
Fibcd1	1.221	0.001762	0.2719	Zfp600	0.8445	0.001923	0.2967	Xlr5a	1.23	0.0009967	0.1538	Trhr	0.8075	0.0007241	0.1124
Snord14e	1.162	0.001792	0.2749					Traj34	1.197	0.001047	0.1606	Rgs4	0.8217	0.000741	0.1143
Gm10354	1.139	0.001825	0.2782					Trbv24	1.239	0.001064	0.1623	Elob	0.8213	0.0007433	0.114
Igf2	1.113	0.001858	0.2815					Olfr1373	1.221	0.001065	0.1614	Gm3973	0.8171	0.000748	0.114
Ighv5-6	1.215	0.001954	0.2943					Gm2016	1.247	0.001086	0.1635	Prelp	0.8215	0.0007606	0.1152
D630041G03Rik	1.219	0.001978	0.2962					Gm14525	1.205	0.001112	0.1665	1700028P14Rik	0.8548	0.0007668	0.1155
Mir26a-2	1.219	0.001983	0.2952					Gm29721	1.235	0.001136	0.1691	Zfp983	0.8166	0.000768	0.115
Vat1l	1.168	0.002007	0.2969					Gja10	1.195	0.001142	0.169	Rab37	0.8213	0.0007867	0.1171
Ifna15	1.166	0.002015	0.2965					Igkv10-94	1.176	0.001153	0.1696	Sst	0.8203	0.0007959	0.1178
Taar2	1.216	0.00202	0.2955					Igkv4-54	1.196	0.001178	0.1723	Zfp868	0.8324	0.0008031	0.1182
Snord53	1.09	0.002027	0.2948					Mir362	1.225	0.001194	0.1736	Olfr434	0.8509	0.000807	0.118
Igkv4-70	1.21	0.00206	0.2979					4631405K08Rik	1.238	0.001199	0.1734	Scgb2b7	0.8471	0.000824	0.1198
								Vmn2r31	1.152	0.001214	0.1745	Dubr	0.8438	0.0008607	0.1245
								Vmn1r221	1.204	0.001225	0.1752	Gm15418	0.8283	0.0008617	0.1239
								Trav15-2-dv6-2	1.233	0.001227	0.1744	Defb11	0.843	0.0008623	0.1233
								Olfr318	1.234	0.001274	0.1802	Opalin	0.8456	0.0008801	0.1251
								Gm53	1.223	0.001277	0.1795	Gm3173	0.8356	0.0008843	0.125
								Igkv5-37	1.239	0.001314	0.1837	Nme5	0.8291	0.0008887	0.1249
								Gm21310	1.182	0.001336	0.1857	Vmn2r-ps60	0.8586	0.0009017	0.1261
								Snora47	1.189	0.001337	0.1848	Plagl1	0.8203	0.0009101	0.1265
								1700030F04Rik	1.215	0.00135	0.1857	Gm31898	0.8249	0.0009125	0.1262
								Snora3	1.088	0.001354	0.1852	Cfap65	0.8299	0.0009166	0.1261
								Ifna2	1.231	0.001356	0.1845	Zfp459	0.8262	0.0009423	0.1289
								Tlr11	1.234	0.001371	0.1855	Plp2	0.8283	0.0009695	0.1319
								Mir568	1.181	0.001381	0.1858	Mir6360	0.8365	0.001004	0.1358
								Gm27017	1.23	0.00142	0.1901	Lipo2	0.8201	0.001013	0.1364

Table 2. continued

n-3 sufficient down-regulated genes				n-3 sufficient up-regulated genes				n-3 deficient down-regulated genes				n-3 deficient down-regulated genes			
GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP
								Gm32137	1.2	0.001431	0.1906	Snord42a	0.8233	0.001037	0.1389
								Gm35573	1.226	0.001448	0.1918	Tmem163	0.8316	0.001045	0.1391
								Gm34111	1.198	0.001453	0.1915	Mrps36	0.8294	0.001057	0.14
								Trdv2-1	1.257	0.001465	0.1921	Efhc1	0.8294	0.001075	0.1417
								Gm5169	1.211	0.001474	0.1922	Myoc	0.8315	0.001102	0.1444
								Fmod	1.031	0.001494	0.1939	Gm8050	0.8345	0.001114	0.1453
								Btbd35f17	1.049	0.001505	0.1943	Arc	0.9401	0.001115	0.1447
								Gm38408	1.229	0.001528	0.1962	Amotl1	0.8286	0.001138	0.147
								Mir3109	1.226	0.001532	0.1957	Dab2	0.8344	0.001159	0.1489
								Snord52	1.206	0.001544	0.1963	Frmpd3	0.8294	0.001168	0.1493
								Vmn1r125	1.192	0.00156	0.1973	S100a11	0.8251	0.001168	0.1485
								Trav7d-4	1.208	0.001592	0.2004	Atp6ap1l	0.9009	0.001188	0.1503
								Gm12409	1.22	0.001594	0.1997	Gm31863	0.8651	0.001198	0.1508
								Traj54	1.228	0.001597	0.199	Snord49a	0.832	0.001203	0.1507
								Mir6975	1.213	0.001618	0.2007	Gas5	0.8375	0.001256	0.1566
								Magea5	1.228	0.001626	0.2007	Bmp6	0.8489	0.001257	0.1559
								Traj28	1.209	0.001646	0.2022	Ephx1	0.8343	0.001263	0.1559
								Mir7035	1.223	0.001669	0.2041	LOC102639037	0.8689	0.001268	0.1557
								Trav7d-3	1.226	0.001708	0.2078	Enkur	0.8308	0.001277	0.1561
								Defa-ps12	1.222	0.001741	0.2108	Pcdhga2	0.8342	0.001286	0.1564
								Satb2	1.223	0.001741	0.2098	Efhd1	0.8402	0.001286	0.1557
								Mir7215	1.22	0.00176	0.211	Mt2	0.8341	0.001314	0.1584
								Mir7226	1.228	0.00177	0.2113	Gm8702	0.8904	0.00132	0.1583
								Olfr1229	1.215	0.00177	0.2103	Mest	0.8365	0.00133	0.1587
								Gm20063	1.21	0.001772	0.2096	Slc17a6	0.8322	0.001355	0.161
								Igkv4-57	1.192	0.001795	0.2113	Stoml3	0.9044	0.001356	0.1604
								Gm7682	1.21	0.001816	0.2128	Nr2f2	0.8538	0.00138	0.1624
								Vmn2r43	1.121	0.001884	0.2197	Gm6309	0.8444	0.001394	0.1633
								Olfr1321	1.227	0.001886	0.2189	Prp2	0.8321	0.0014	0.1632
								WITHDRAWN BY NCBI (LOC102637484)	1.216	0.00189	0.2184	Iqcj	0.8315	0.00141	0.1636
								Snord118	1.225	0.001894	0.2179	Olfr97	0.8468	0.001462	0.1689
								Mir3103	1.151	0.001904	0.218	Uqcr10	0.8419	0.001503	0.1729
								4930488B22Rik	1.225	0.001932	0.2202	1700094D03Rik	0.878	0.001509	0.1728
								Olfr780	1.221	0.001972	0.2238	Cdh13	0.8345	0.001515	0.1727
								Akr1c18	1.224	0.001974	0.2231	Crym	0.8486	0.001536	0.1743
								Ttc30a2	1.184	0.001976	0.2222	Ighv1-19-1	0.8458	0.001541	0.1741
								Gm5622	1.196	0.002023	0.2265	Pcolce	0.8494	0.001557	0.1751
								Mir344d-2	1.207	0.002107	0.2349	Slc7a10	0.8281	0.001583	0.1773
								Vmn1r41	1.163	0.002114	0.2347	Slc12a2	0.8443	0.001592	0.1767
								Mir222	1.211	0.002136	0.2361	Cfap206	0.8484	0.001594	0.1761
								Snord14e	1.23	0.002192	0.2412	Zdhhc22	0.8335	0.00167	0.1837
								Olfr467	1.213	0.002239	0.2453	Mlf1	0.8375	0.001697	0.1859
								Olfr1335	1.231	0.002239	0.2443	Wdr6	0.8355	0.001709	0.1864
								Olfr299	1.212	0.00224	0.2433	Gm3187	0.9165	0.00172	0.1868
								Olfr170	1.145	0.002278	0.2464	Vcam1	0.8431	0.001727	0.1867
								Mir6961	1.216	0.002301	0.2478	Rhox4f	0.8404	0.001765	0.19
								Mir466l	1.212	0.002302	0.2469	Mir218-1	0.8657	0.001827	0.1959

Table 2. continued

n-3 sufficient down-regulated genes				n-3 sufficient up-regulated genes				n-3 deficient down-regulated genes				n-3 deficient down-regulated genes			
GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP
								Mageb4	1.219	0.002321	0.2478	Trav9-4	0.8739	0.00183	0.1954
								Igkv8-24	1.211	0.002349	0.2498	Gm13363	0.8413	0.001837	0.1953
								Mir7674	1.188	0.002356	0.2495	Gm7846	0.8365	0.001854	0.1963
								Olfr1250	1.167	0.002357	0.2485	Vmn1r79	0.8396	0.001921	0.2026
								Vmn1r113	1.148	0.002357	0.2475	Zfp185	0.8429	0.001942	0.2039
								Gm8221	1.214	0.002361	0.2469	Rps4l	0.8527	0.002006	0.2098
								Gm29993	1.22	0.002381	0.248	Fras1	0.8445	0.002015	0.2098
								Igkv4-73	1.159	0.002406	0.2496	Mir299b	0.8949	0.002041	0.2117
								1700010D01Rik	1.217	0.002411	0.2491	Cxcr4	0.8447	0.002052	0.2119
								Gm17308	1.216	0.002413	0.2482	Stra6	0.8497	0.002067	0.2127
								Ighv1-61	1.197	0.002432	0.2491	B930018H19Rik	0.8368	0.00211	0.2161
								Olfr1356	1.212	0.002446	0.2496	Sfrp1	0.8485	0.002154	0.2198
								Bmp3	1.176	0.002476	0.2517	Rsc1a1	0.8454	0.002187	0.2223
								Gm31250	1.178	0.002486	0.2517	Gm11677	0.8477	0.002205	0.2232
								Mir704	1.212	0.002512	0.2532	Mir1264	0.846	0.002225	0.2243
								Omd	1.113	0.002557	0.2567	Cpne7	0.838	0.00224	0.225
								Olfr767	1.171	0.002563	0.2564	Gstm2	0.8464	0.002247	0.2248
								Olfr19	1.214	0.002596	0.2586	Cox8b	0.8502	0.002288	0.2279
								Gm36139	1.21	0.002608	0.2588	Gsr	0.8487	0.002323	0.2305
								Eqtn	1.223	0.002622	0.2592	Tnc	0.8493	0.002323	0.2297
								Cstdc5	1.186	0.002639	0.2599	Trbv13-2	0.8448	0.00233	0.2294
								BC018473	1.2	0.002644	0.2593	Slc16a4	0.8493	0.002354	0.2309
								Iglj2	1.209	0.002644	0.2584	Esp5	0.8436	0.00242	0.2365
								Mir150	1.214	0.002659	0.2588	Otx2os1	0.8478	0.002424	0.2359
								Olfr974	1.213	0.00267	0.2588	Best3	0.8495	0.002433	0.2358
								Olfr1491	1.21	0.00267	0.2579	Dusp18	0.8452	0.002444	0.2361
								Hist1h1d	1.211	0.002694	0.2592	Mid1	0.8514	0.00245	0.2358
								Olfr317	1.209	0.002696	0.2584	Ptgds	0.9537	0.00247	0.2368
								Trdv2-2	1.178	0.002729	0.2606	B2m	0.8645	0.002485	0.2373
								Gm15363	1.217	0.002739	0.2605	B130024G19Rik	0.8455	0.002494	0.2372
								Olfr681	1.191	0.002792	0.2646	Sh3d19	0.8496	0.002505	0.2374
								Carmil1	1.192	0.002793	0.2637	AF067061	0.87	0.002512	0.2372
								Mir3089	1.198	0.002805	0.2638	Cdhr1	0.8493	0.00252	0.2371
								Mir466d	1.212	0.002821	0.2644	Dnah11	0.8681	0.00256	0.2399
								Mir466f-4	1.21	0.002841	0.2653	Adora2b	0.8555	0.00258	0.2409
								Ffar1	1.171	0.002864	0.2665	Serpinb1a	0.8507	0.002612	0.243
								Krtap5-5	1.208	0.002901	0.2689	Abhd2	0.8499	0.002626	0.2434
								AF067063	1.192	0.002925	0.2702	WITHDRAWN BY NCBI (LOC102632416)	0.872	0.002636	0.2434
								Tnfrsf17	1.21	0.002948	0.2713	Scgb2b6	0.8501	0.002646	0.2434
								Zfp97	1.094	0.002959	0.2713	Olfml1	0.849	0.002651	0.2431
								Magea2	1.21	0.00297	0.2713	n-TRtcg5	0.8865	0.002665	0.2434
								Mir195b	1.207	0.003113	0.2833	Chrm5	0.8769	0.002719	0.2475
								Vmn1r183	1.21	0.003156	0.2862	Gm10362	0.8495	0.002721	0.2468
								Tnnt2	1.206	0.003179	0.2873	Cdkn1c	0.8497	0.002729	0.2466
								Cyp11a1	1.198	0.003203	0.2885	Snord16a	0.8336	0.002729	0.2457
								Trav8n-2	1.202	0.003234	0.2902	Sh3bgrl3	0.8522	0.00273	0.245
								Gm14393	1.059	0.003327	0.2975	Ccdc8	0.8929	0.00276	0.2468

Table 2. continued

n-3 sufficient down-regulated genes				n-3 sufficient up-regulated genes				n-3 deficient down-regulated genes				n-3 deficient down-regulated genes			
GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP	GeneSymbol	FC: (Sal/LPS)	*p* value	PFP
								Tcl1b5	1.206	0.003368	0.3001	Epb41l4a	0.8495	0.002827	0.2519
								Gm12887	1.208	0.003377	0.2998	Gm10512	0.8544	0.002883	0.256
								Rnu73b	1.119	0.003389	0.2998	1700020N15Rik	0.9718	0.002898	0.2564
								Olfr462	1.205	0.003393	0.2992	Strip2	0.8678	0.002902	0.2558
								Mir19b-1	1.16	0.003396	0.2984	Gm15127	0.9024	0.002959	0.26
												AW551984	0.8478	0.00297	0.26
												Snord34	0.8467	0.002978	0.2599
												Snord72	0.8603	0.002989	0.2599
												Peg3os	0.8513	0.003025	0.2622
												Dnah6	0.8556	0.003028	0.2615
												Mir872	0.8509	0.003054	0.2628
												Olfr358	0.8465	0.003082	0.2644
												Izumo4	0.8562	0.003116	0.2664
												Tekt1	0.8572	0.003136	0.2672
												Snord111	0.8593	0.003146	0.2672
												Snord22	0.8479	0.003189	0.2699
												AF357399	0.8916	0.003194	0.2694
												4930482G09Rik	0.8486	0.003196	0.2687
												Ankrd34c	0.8683	0.00321	0.269
												Alg14	0.8533	0.003289	0.2747
												Clybl	0.8525	0.003297	0.2745
												Gas6	0.8582	0.003359	0.2787
												Gm38485	0.8491	0.003392	0.2805
												Tnnt1	0.8612	0.003394	0.2797
												Gm9079	0.856	0.003422	0.2812
												Cgnl1	0.8555	0.003435	0.2813
												Serpinb1b	0.85	0.003444	0.2812
												Krtap4-8	0.8488	0.003526	0.2869
												Gm7609	0.8463	0.00361	0.2928
												Cd59a	0.8669	0.003634	0.2938
												Phtf1os	0.8572	0.003636	0.293
												Hist1h3c	0.8739	0.003638	0.2922
												Snord55	0.865	0.003644	0.2918
												Gm9639	0.8732	0.003645	0.291
												Wdr63	0.881	0.003647	0.2902
												Erdr1	0.8559	0.003684	0.2922
												Akr1e1	0.8897	0.003688	0.2916
												Rsph4a	0.8719	0.003714	0.2927
												Gm31606	0.9302	0.003792	0.298
												Slc38a3	0.8595	0.003828	0.2999
												Nupr1	0.8735	0.003831	0.2992

The Proportion of False Positives prediction (PFP, equivalent in theory of false discovery rate or FDR) is provided by RankProduct. The pfp score gives the rate of type I errors in null hypothesis testing when conducting multiple comparisons.

Table 3. Common differentially expressed genes (DEGs) between n-3 sufficient and n-3 deficient group.

Common DEGs (LPS vs Saline) up-deficient vs up-Sufficient	Common DEGs (LPS vs Saline) down-deficient vs down-Sufficient	Common DEGs (LPS vs Saline) up-deficient vs down-Sufficient	Common DEGs (LPS vs Saline) Down-deficient vs up-Sufficient
1810020O05Rik	1500004A13Rik	Ttr	Gm31663
Cwc22	Gm15143	Clic6	Trav9n-4
Gm3435	Trav13n-1	1500015O10Rik	Ighv1-37
Dynlt1f	Snora44	F5	Masp2
Btbd35f12	LOC102636873	Kl	Igkv4-72
Gm30181	Traj1	Enpp2	Gm32970
Gm5797	Scgb2b12	Lbp	Mir101c
Gm8702	Gm14147	Igf2	Olfr205
Gm5945	Snord45b	Gm26808	Jmjd7
Olfr153	Traj39	Folr1	Fggy
Luzp4	Speer4e	Snora35	Zfp934
Mageb5	Gm35974	Tmem72	Rxfp1
AV320801	Traj14	Prlr	LOC105245735
Bcl2a1b	Trbj1-5	Ace	Gapdh-ps15
Vmn2r-ps60	Gm14393	Sulf1	Gm5169
Trav13-4-dv7	LOC102633225	Igfbp2	Gm15363
Gsr	Igkv4-79	Vat1l	Zfp97
LOC102632416	Snora47	Prkcd	
Gm3973	Gm38474	Zic1	
Gm10087	Ighv9-2	Mir1912	
Gm8050	Snord71	Gpx8	
Snord57	Gm16427	Slc4a5	
Gm38481	Ighv1-20	Sostdc1	
Mndal	Tdpoz3	Syt9	
Gm13249	Xlr3b	Calml4	
Atp6v0c-ps2	Vmn2r44	Atp5g1	
Gm13034	Trav15-2-dv6-2	Gm3409	
Zfp33b	LOC100502592	Tgtp2	
Snord14c	Fmod	Slitrk6	
Gm3164	Gm20063	Baiap2l1	
Snord68	Ccl28	Defb11	
	Igkv6-29	Crym	
	Gm32783	Gm3187	
	Snord14e	Mid1	
	Ifna15	1700020N15Rik	
	Taar2	Akr1e1	

in the post-natal period. (6) Microbial composition and gut inflammatory molecules correlated with neurobiological outcome in pups. This study must now be extended to female mice, since most, if not all, the parameters studied are sensitive to sex, and to increase its clinical relevance [74, 111–113].

We show that MIA reveals spatial memory deficits and hyper-locomotion behavior only when combined with low n-3 PUFA intake. This is contradictory to previous studies that showed a significant effect of MIA alone on behavior of adult mice, including learning and memory abilities [8, 114–124]. We however confirmed a previous report from our group in which n-3 PUFA deficiency induces memory deficits in MIA-exposed mice [28]. As a plausible explanation for this discrepancy, our control animals are fed with an n-3 sufficient diet, whose composition is distinct from standard chow (less MUFA, more PUFA and higher n-6/n-3 ratio in the standard chow vs n-3 sufficient diet) [125]. Moreover, the type and the dose of MIA-inducing agent, as well as the embryonic age of exposure, are also key for long-term behavioral deficits [8].

One cannot rule out that MIA-induced maternal care defects is a plausible cause for the long term deficits observed in the offspring. Indeed, it was previously shown that the adoption of control neonates by surrogate rearing mothers, previously exposed to MIA during pregnancy, is sufficient to trigger cognitive deficits in the fostered offspring [13, 109, 126–129]. Nonetheless, unlike other studies using a similar approach to ours, i.e., one single i.p. administration of LPS at E17 in mice, we could not find any major effects of MIA in n-3 sufficient mice, especially on memory and anxiety [11, 80, 82, 118, 130, 131]. This suggests intrinsic differences between standard chow-fed (as used in previous studies) and n-3 PUFA sufficient-fed mice and questions the underlying mechanisms. We previously showed that the brain fatty acid composition of mice fed either a standard or n-3 sufficient diet is quite similar [125]. However, the two diets vary in total saturated fat, monounsaturated, PUFAs, LA/ALA ratio, proteins, carbohydrates, and total lipids. Hence, aspects other than brain fatty acid composition are likely to explain differences between both groups, such as cell energy metabolism, lipid composition at the cellular resolution or production of lipid derivatives. This remains to be addressed.

We evaluated the effect of dietary n-3 PUFA deficiency combined with MIA on gut inflammation and microbiota composition, as a plausible mechanism underlying behavioral alterations. α-divesity was decreased by both interventions at PND14 and PND21. Similar observations were made in patients with neurodevelopmental diseases such as ADHD [132]. Of note, bacteria from the *Prevotellaceae* family were less abundant in n-3 deficient mice while *Akkermansia* was increased. While we must be cautious in making comparisons between human and mice findings regarding gut microbiota composition [133], similar differences have been observed between autistic children and controls of similar age [134, 135]. Moreover, increased levels of *Prevotella* following a fecal material transfer in autistic patients have been associated with improvements of autistic symptoms [136], while some studies report that *Akkermansia* levels are elevated in multiple sclerosis (MS) or Parkinson's Disease patients' [137–139]. We also observe an elevation of *Enterobacteriaceae* in n-3 deficient mice, which was similarly found to be increased in autistic children [140] and decreased after n-3 PUFA supplementation [141]. Our work is also in line with two studies demonstrating that maternal n-3 PUFA deficiency induces compositional and functional disturbances to the gut microbiome, closely associated with long term behavioral consequences in the offspring [142, 144].

We show for the first time that low maternal n-3 PUFA intake alone or combined to MIA alters gut structure and physiology during post-natal development in a time-dependent manner. Previous study reported altered intestinal permeability in the offspring in a poly(I:C) model of MIA [59]. Several studies reveal the impact of n-3 PUFAs on gut health (intestinal permeability and gut innervation) [143, 145–150]. The gut immune response, tightly related to gut permeability, was exacerbated in n-3 deficient mice at PND14, while at PND21, it was enhanced in n-3 sufficient mice. More studies are needed to understand whether and how the delay of response in n-3 sufficient mice explains their protection in terms of long-term cognitive abilities. A recent study highlighted the prominent role of T helper 17 (T_H17)-derived IL-17A in neurodevelopmental abnormalities in the offspring of pregnant mothers undergoing MIA [61]. In our study, we observed a general overactivation of the gut inflammatory response in MIA-exposed offspring during CNS development. Interestingly, clinical studies report defects in gut permeability and inflammatory response in ASD and schizophrenic patients [60, 107, 133], suggesting that the

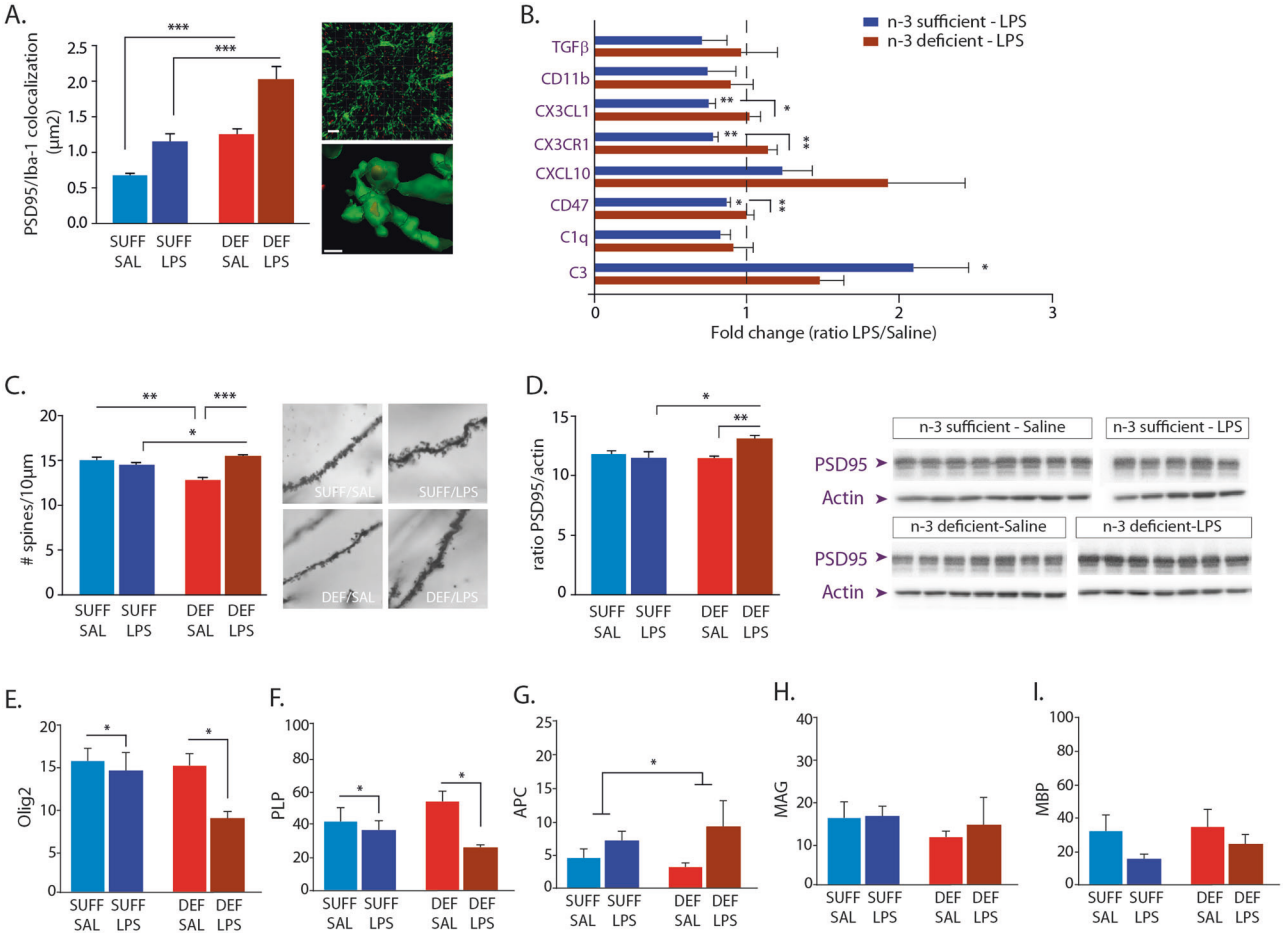


Fig. 3 Effect of n-3 PUFA deficiency and MIA on microglia-neuron crosstalk pathways, spine density, oligodendrocyte and myelin protein expression. All graphs show Means ± SEM. **a** Colocalization of Iba-1 and PSD95 proteins immunoreactivity in the CA1 region of the hippocampus of PND14 pups. Representative confocal image of Iba-1 (green) PSD95 (red) costaining (Top panel: scale bar = 10 µm) and Imaris 3D reconstruction (Bottom panel, scale bar = 1 µm). $N = 72–122$. Kruskal–Wallis test followed by Mann–Whitney comparisons; n-3 deficient-Saline vs n-3 sufficient-Saline, ***$p < 0.0001$; n-3 deficient-LPS vs n-3 sufficient-LPS, ***$p < 0.0001$. **b** qRT-PCR quantification of microglia-neuron interaction mRNA markers in the hippocampus of PND14 mice (data normalized to the saline group, dotted line). $N = 4–6$. Kruskal–Wallis test followed by Mann–Whitney comparisons; *$p < 0.05$, **$p < 0.01$ (all comparisons in Table S2). **c** Quantification and representative images of Golgi staining spine density in the CA1 region of the hippocampus at PND28. $N = 8–21$. Kruskal–Wallis test followed by Mann–Whitney comparisons; n-3 deficient-Saline vs n-3 deficient-LPS, ***$p < 0.0001$; n-3 deficient-Saline vs n-3 sufficient-Saline, **$p = 0.001$; n-3 deficient-LPS vs n-3 sufficient-LPS, *$p = 0.025$. **d** Western blot-based quantification and representative images of PSD95 protein expression in the hippocampus of PND28 mice. $N = 4–8$. Kruskal–Wallis test followed by Mann–Whitney comparisons; n-3 deficient-Saline vs n-3 deficient-LPS, **$p = 0.004$; n-3 deficient-LPS vs n-3 sufficient-LPS, *$p = 0.03$. Quantification of Olig2 (**e**), PLP (**f**), APC (**g**), MAG (**h**) and MBP (**i**) immunoreactivity in the hippocampus of PND14 mice. $N = 4–7$. Two-way ANOVA. Olig2: diet effect, $F(1,20) = 3.48$, $p = 0.08$; MIA effect, $F(1,20) = 4.78$, $p = 0.041$. *PLP*: MIA effect, $F(1,22) = 5.01$, $p = 0.036$. *APC*: diet effect, $F(1,17) = 4.96$, $p = 0.0397$.

double environmental insult recapitulates some aspects of these diseases.

At PND14, we found that levels of several members of the *Lachnospiraceae* family correlate with and myelin markers and markers of gut immune reactivity. Recent study highlighted, *Ruminococcus Gnavus* as a central player in Crohn's disease. These bacteria have been shown to stimulate gut inflammation through the release of specific metabolites [150]. *Lachnospiraceae* is decreased in MS patients [151], who are known to exhibit an altered T-cell response together with alterations of the intestinal barrier permeability [152]. Contrary to our own data, Hsiao et al. showed that MIA increases *Lachnospiraceae* [59]. The discrepancy could result from different timelines of analysis: we analyzed microbiota composition in PND14-21 mice, while Hsiao et al. studied its composition after weaning. It could also be explained by the different MIA protocols used: bacterial at E17 vs viral mimetic at E12.5. At the genus level, we observed opposite variation of members of this family. Linking global *Lachnospiraceae* levels with biological outcomes appears to be inappropriate, as the family comprises of a large number of distinct bacteria. It could explain why a human study of associations between the levels of Lachnospiraceae and neurological diseases, such as depression, also found conflicting results [153].

N-3 PUFA deficiency exacerbated MIA-induced defects in microglia-neuron interactions, while microglial density, phenotype and inflammatory activity were unaffected in pups. This confirms most previous reports in which no effect of MIA on microglial density were ever observed [34, 116, 154–158]. We previously demonstrated that the proinflammatory cytokine expression is exacerbated in both maternal and embryonic brains of the n-3 deficient group [28], which is no longer the case at PND14. However, increased number of Iba1/PSD95 positive cells were measured in the hippocampus of n-3 deficient offspring at PND14, suggestive of synaptic pruning [29]. In line with these data, the hippocampal synaptic density was decreased at PND28. These

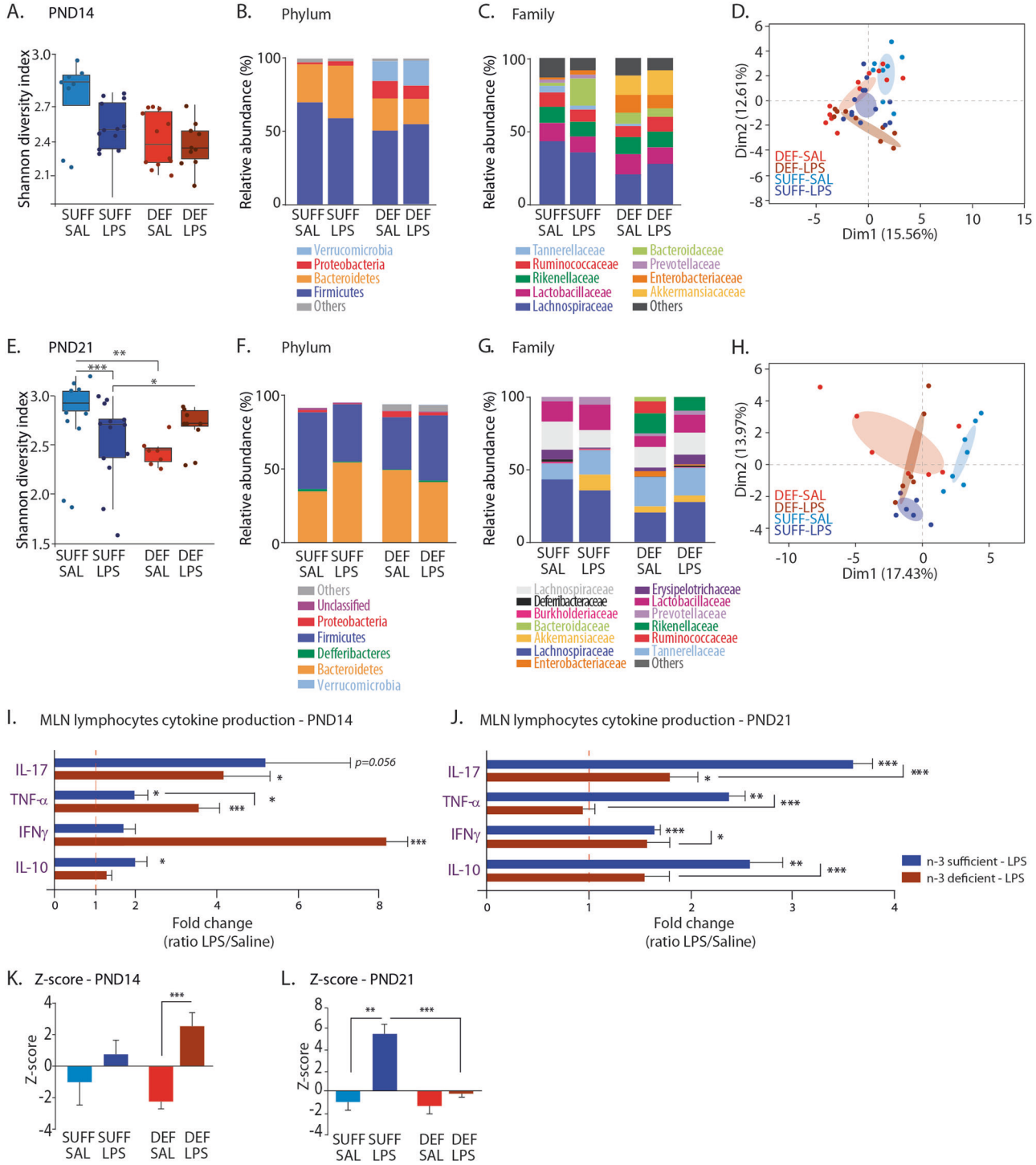


Fig. 4 Effect of n-3 PUFA deficiency and MIA on gut microbiota composition at PND14 and PND21. All graphs show Means ± SEM. **a** 16S rRNA-sequencing-based alpha diversity analysis of the microbiota, measured by Shannon index in PND14 mice. $N = 8–12$. Two-way ANOVA: diet effect, $F(1,39) = 12.76$, $p < 0.001$; MIA effect, $F(1,39) = 5.39$, $p = 0.026$. Bacteria phyla (**b**) and family (**c**) observed in all experimental groups at PND14. **d** PCA of all subjects at PND14. Confidence ellipses appear around each group. **e** 16S rRNA-sequencing-based alpha diversity analysis, measured by Shannon index in PND21 mice. $N = 8–13$. Two-way ANOVA: n-3 sufficient-Saline vs n-3 sufficient-LPS, ***$p = 0.0005$; n-3 sufficient-LPS vs n-3 deficient-LPS, *$p = 0.019$; n-3 sufficient-Saline vs n-3 deficient-Saline, **$p = 0.0078$. Bacteria phyla (**f**) and family (**g**) observed in all experimental groups at PND21. **h** PCA of all subjects at PND14. Confidence ellipses appear around each group. Quantification of MLN lymphocytes cytokine release measured by ELISA at PND14 (**i**) and PND21 (**j**). $N = 6–15$; Kruskal–Wallis test followed by Mann–Whitney comparisons; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (all comparisons in Table S2). Z-score of T cells inflammatory reactivity in PND14 (**k**) and PND21 (**l**) mice. $N = 7–15$. Kruskal–Wallis test followed by Mann–Whitney comparisons; *PND14:* n-3 deficient-Saline vs n-3 deficient-LPS, ***$p < 0.001$. *PND21*: n-3 sufficient-Saline vs n-3 sufficient-LPS, **$p = 0.0011$, n-3 sufficient-LPS vs n-3 deficient-LPS, ***$p = <0.0004$.

Table 4. 16S rRNA-sequencing-based bacteria genus observed in all experimental groups at PND14.

PND14	Mean ± SEM	n-3 sufficient-Saline	n-3 sufficient-LPS	n-3 deficient-Saline	n-3 deficient-LPS	Kruskal–Wallis (*p* value)	SUFF SAL vs. SUFF LPS	DEF SAL vs. DEF LPS	SUFF SAL vs. DEF SAL	SUFF LPS vs. DEF LPS
Acetatifactor	Mean	0.08	0.30	0.00	0.08	<0.01	NS	NS	NS	<0.05
	sem	0.05	0.12	0.00	0.05					
Akkermansia	Mean	0.00	0.02	13.32	16.88	<0.001	NS	NS	<0.001	<0.05
	sem	0.00	0.02	4.91	6.15					
Alistipes	Mean	10.12	8.06	9.94	5.94	NS	NS	NS	NS	NS
	sem	1.21	2.19	1.69	2.19					
Alloprevotella	Mean	1.53	2.64	0.00	0.01	<0.05	NS	NS	<0.01	NS
	sem	1.50	2.00	0.00	0.01					
Anaerostipes	Mean	2.03	1.64	1.02	1.72	<0.01	NS	NS	<0.01	NS
	sem	0.23	0.23	0.22	1.00					
Anaerotruncus	Mean	0.03	0.13	0.17	0.22	<0.05	NS	NS	NS	NS
	sem	0.01	0.05	0.07	0.06					
Anaerovorax	Mean	0.13	0.20	0.13	0.66	NS	NS	NS	NS	NS
	sem	0.04	0.06	0.03	0.28					
ASF356	Mean	0.05	0.01	0.06	0.00	<0.01	<0.01	NS	NS	NS
	sem	0.01	0.01	0.03	0.00					
Bacteroides	Mean	2.29	18.51	7.68	5.56	<0.01	<0.001	NS	NS	<0.01
	sem	0.98	2.44	2.88	2.90					
Blautia	Mean	23.43	20.83	9.21	13.79	<0.05	NS	NS	<0.01	NS
	sem	4.55	3.75	1.43	2.85					
Butyricicoccus	Mean	0.03	0.08	0.02	0.40	<0.001	NS	<0.001	NS	<0.01
	sem	0.02	0.03	0.01	0.12					
Dorea	Mean	0.01	0.27	0.50	0.96	NS	NS	NS	<0.05	NS
	sem	0.00	0.12	0.20	0.50					
Escherichia-Shigella	Mean	1.50	2.93	11.91	9.11	<0.01	NS	NS	<0.01	<0.05
	sem	0.98	0.91	3.23	2.42					
Eubacterium copro	Mean	1.03	0.27	0.30	0.39	<0.05	<0.05	NS	<0.01	NS
	sem	0.27	0.10	0.20	0.17					
Hespellia	Mean	1.32	0.33	0.28	0.58	<0.05	<0.01	NS	<0.01	NS
	sem	0.34	0.10	0.07	0.20					
Lachnoclostridium	Mean	0.24	0.28	0.12	0.40	NS	NS	<0.05	NS	NS
	sem	0.08	0.08	0.04	0.14					
Lachnospiraceae NK4A136	Mean	0.34	0.09	0.24	0.06	<0.05	<0,01	NS	NS	NS
	sem	0.10	0.02	0.10	0.02					
Lachnospiraceae UCG-008	Mean	2.08	1.00	0.30	0.12	<0.001	<0,01	NS	<0.001	NS
	sem	0.28	0.34	0.14	0.06					
Lactobacillus	Mean	12.31	10.10	13.67	11.37	NS	NS	NS	NS	NS
	sem	2.03	1.16	2.88	2.41					
Marvinbryantia	Mean	0.00	0.33	0.00	0.01	<0.01	<0,05	NS	NS	<0.05
	sem	0.00	0.12	0.00	0.01					
Oscillibacter	Mean	0.53	0.38	0.73	0.23	<0.05	NS	<0.01	NS	NS
	sem	0.09	0.15	0.16	0.12					
Parabacteroides	Mean	4.28	2.63	1.35	0.01	<0.001	NS	NS	<0.05	<0.001
	sem	1.74	0.95	0.79	0.00					
Prevotellaceae UCG-001	Mean	0.50	0.00	0.00	0.00	NS	NS	NS	NS	NS
	sem	0.50	0.00	0.00	0.00					
Rikenellaceae RC9	Mean	0.93	2.27	1.75	4.79	NS	NS	NS	NS	NS
	sem	0.59	1.05	0.72	3.92					
Roseburia	Mean	4.00	3.20	4.64	3.31	NS	NS	NS	NS	NS
	sem	0.73	1.14	1.11	1.05					
Ruminiclostridium	Mean	2.74	2.86	2.60	2.16	NS	NS	NS	NS	NS
	sem	0.84	0.42	0.53	0.73					
Ruminiclostridium 9	Mean	1.55	1.51	0.74	2.32	<0.05	NS	<0.01	<0.05	NS
	sem	0.27	0.31	0.13	0.40					
Ruminococcus GG	Mean	1.47	1.34	0.46	1.72	<0.01	NS	<0.01	<0.001	NS
	sem	0.20	0.28	0.06	0.48					
Staphylococcus	Mean	1.08	0.73	0.57	0.30	NS	NS	NS	NS	NS
	sem	0.19	0.21	0.15	0.08					
Stomatobaculum	Mean	0.34	0.09	0.54	0.10	NS	NS	NS	NS	NS
	sem	0.11	0.03	0.18	0.05					
Streptococcus	Mean	0.57	0.50	0.74	0.34	NS	NS	NS	NS	<0.05
	sem	0.09	0.05	0.22	0.06					
Tyzzerella	Mean	0.86	0.68	0.51	1.32	NS	NS	NS	NS	NS
	sem	0.19	0.12	0.16	0.49					
Unclassified	Mean	17.49	12.30	12.66	10.85	NS	NS	NS	NS	NS
	sem	1.99	1.37	2.11	1.08					

Data are presented as mean ± SEM.

Table 5. 16S rRNA-sequencing-based bacteria genus observed in all experimental groups at PND21.

PND21	Mean ± SEM	n-3 sufficient-Saline	n-3 sufficient-LPS	n-3 deficient-Saline	n-3 deficient-LPS	Kruskal–Wallis (p value)	SUFF SAL vs. SUFF LPS	DEF SAL vs. DEF LPS	SUFF SAL vs. DEF SAL	SUFF LPS vs. DEF LPS
Akkermansia	Mean	0.01	0.01	4.35	4.56	<0.05	NS	<0.01	NS	NS
	sem	0.00	0.00	1.86	2.40					
Alistipes	Mean	5.82	1.07	5.31	4.92	<0.05	<0.01	NS	NS	<0.001
	sem	0.90	0.58	1.11	0.56					
Alloprevotella	Mean	2.94	9.60	1.18	2.34	<0.001	<0.01	<0.01	NS	<0.001
	sem	1.02	1.82	1.06	0.66					
Anaeroplasma	Mean	0.14	0.00	0.00	0.00	<0.001	<0.05	NS	<0.01	NS
	sem	0.07	0.00	0.00	0.00					
Anaerostipes	Mean	1.42	0.83	0.60	0.71	<0.05	NS	NS	<0.01	NS
	sem	0.22	0.15	0.11	0.10					
Anaerotruncus	Mean	0.10	0.64	0.25	0.26	<0.05	<0,01	NS	NS	<0,05
	sem	0.04	0.19	0.10	0.11					
ASF356	Mean	0.15	0.04	0.28	0.06	<0.05	NS	<0.05	NS	NS
	sem	0.05	0.03	0.09	0.03					
Bacteroides	Mean	9.07	29.86	20.06	19.21	<0.01	<0.001	NS	<0.05	<0.05
	sem	2.43	3.68	3.11	3.73					
Bilophila	Mean	0.32	0.02	0.23	0.29	<0.05	<0.05	NS	NS	<0.01
	sem	0.11	0.02	0.07	0.12					
Blautia	Mean	5.07	0.87	3.81	5.63	<0.05	<0.01	NS	NS	NS
	sem	0.76	0.28	1.03	1.58					
Dorea	Mean	0.31	0.67	0.35	0.47	NS	NS	NS	NS	NS
	sem	0.10	0.22	0.15	0.10					
Enterococcus	Mean	0.00	0.01	0.23	0.01	<0.05	NS	NS	<0.01	NS
	sem	0.00	0.01	0.17	0.01					
Erysipelatoclostridium	Mean	0.00	0.00	0.26	0.60	<0.001	NS	<0.05	NS	<0.01
	sem	0.00	0.00	0.18	0.22					
Escherichia-Shigella	Mean	0.04	0.07	3.58	0.86	<0.001	<0.05	NS	<0.001	<0.05
	sem	0.01	0.02	1.96	0.40					
Eubacterium coprostanoligenes group	Mean	0.05	0.16	0.00	0.00	NS	NS	NS	NS	NS
	sem	0.03	0.16	0.00	0.00					
Faecalibaculum	Mean	6.78	0.69	2.05	5.96	NS	NS	NS	NS	<0.05
	sem	2.14	0.25	0.90	2.54					
Hespellia	Mean	1.26	0.82	0.36	0.35	<0.01	NS	NS	<0.01	NS
	sem	0.17	0.33	0.13	0.08					
Lachnoclostridium	Mean	1.11	0.80	1.53	1.57	NS	NS	NS	NS	NS
	sem	0.16	0.11	0.20	0.38					
Lachnospiraceae NK4A136 group	Mean	1.60	0.76	0.40	0.91	<0.01	<0.05	NS	<0.001	NS
	sem	0.26	0.39	0.12	0.30					
Lachnospiraceae UCG-008	Mean	0.50	0.14	0.30	0.16	<0.05	<0.05	NS	NS	NS
	sem	0.14	0.14	0.09	0.13					
Lactobacillus	Mean	14.41	15.90	7.42	12.33	NS	NS	NS	<0.01	NS
	sem	1.76	4.54	1.99	3.02					
Mucispirillum	Mean	1.85	0.52	0.39	0.98	<0.001	<0.01	<0.05	<0.001	NS
	sem	0.15	0.32	0.15	0.12					
Odoribacter	Mean	0.23	0.00	0.00	0.00	NS	NS	NS	NS	<0.01
	sem	0.15	0.00	0.00	0.00					
Olsenella	Mean	0.13	0.01	0.05	0.22	NS	NS	NS	NS	NS
	sem	0.05	0.00	0.02	0.12					
Oscillibacter	Mean	1.54	0.12	0.51	0.43	<0.001	<0.001	NS	<0.01	<0.05

Table 5. continued

PND21	Mean ± SEM	n-3 sufficient-Saline	n-3 sufficient-LPS	n-3 deficient-Saline	n-3 deficient-LPS	Kruskal–Wallis (p value)	SUFF SAL vs. SUFF LPS	DEF SAL vs. DEF LPS	SUFF SAL vs. DEF SAL	SUFF LPS vs. DEF LPS
	sem	0.25	0.03	0.11	0.10					
Parabacteroides	Mean	6.10	6.48	10.97	4.89	NS	NS	NS	NS	NS
	sem	1.28	1.29	5.09	1.16					
Parasutterella	Mean	1.15	0.21	0.07	0.38	<0.001	<0.001	<0.05	<0.001	NS
	sem	0.17	0.04	0.03	0.20					
Prevotellaceae UCG-001	Mean	0.01	0.00	0.59	0.39	<0.01	NS	NS	<0.01	<0.05
	sem	0.01	0.00	0.27	0.23					
Rikenellaceae RC9 gut group	Mean	4.27	6.01	8.68	6.76	NS	NS	NS	NS	NS
	sem	0.65	1.60	2.34	1.46					
Roseburia	Mean	1.69	4.40	2.11	2.60	<0.05	<0.01	NS	NS	<0.05
	sem	0.30	0.78	0.40	0.44					
Ruminiclostridium	Mean	2.92	1.74	2.00	1.62	NS	<0,05	NS	NS	NS
	sem	0.41	0.14	0.43	0.30					
Ruminiclostridium 9	Mean	2.58	0.44	2.74	1.19	<0.001	<0.001	<0.05	NS	<0.01
	sem	0.32	0.12	0.58	0.22					
Ruminococcus 1	Mean	1.32	1.44	2.19	2.91	<0.01	NS	NS	NS	<0.05
	sem	0.20	0.32	0.36	0.43					
Ruminococcus gnavus group	Mean	1.26	1.04	1.10	0.85	NS	NS	NS	NS	NS
	sem	0.16	0.26	0.17	0.10					
Stomatobaculum	Mean	0.05	0.02	0.04	0.11	NS	NS	NS	NS	NS
	sem	0.03	0.02	0.03	0.11					
Tyzzerella	Mean	1.02	0.70	0.51	0.48	NS	NS	NS	NS	NS
	sem	0.20	0.11	0.11	0.05					
Unclassified	Mean	14.08	9.02	9.35	8.60	<0.001	<0.001	NS	<0.01	NS
	sem	0.49	1.09	1.27	1.17					

Data are presented as mean ± SEM.

findings reinforce our previous observations that early-life n-3 PUFA dietary deficiency alters post-natal microglia phenotype and phagocytic activity in the hippocampus [44, 77, 159]. However, while n-3 PUFA deficiency combined with MIA further increased Iba-1/PSD95 colocalization at PND14, at PND28 the spine density in this group was higher compared with that of saline-injected n-3 deficient mice. These data suggest that the regulation of spine density relies on microglia-independent mechanisms in the n-3 deficient/LPS mice. Microglial phagocytic capacity might be overridden in that context, or compensatory mechanisms are put in place between PND14 and PND28, leading to a significant increase in spine density. More experiments are required to test the functional state of the excess spines of n-3 deficient/LPS mice at PND28 and how it relates to behavioral deficits observed in these mice in adulthood. Our study also revealed an impact of n-3 PUFA deficiency and MIA on microglia-neuron crosstalk, confirming a previous report describing a correlation between spine density and CX3CR1 expression in the hippocampus of MIA-exposed mice [160]. The crucial pathways for this interaction are likely to be dysregulated in our experimental context (complement cascade, fractalkine pathway, CD47) [29, 30, 161]. This concurs with previous studies showing that n-3 PUFAs modulate spine density and microglial activity [20, 44, 77, 162–164].

In conclusion, our study is the first to examine potential mechanisms underlying the link between low n-3 PUFA intake and MIA, including interactions between enteric microbiota and the CNS. We uncover a correlative relationship between diet- and MIA-induced gut alterations and deleterious neurobiological outcomes. We also highlight the post-natal period as a vulnerable time window for perinatal dietary and immune stress. We finally demonstrate the long-lasting effect of these stressors, both at the transcriptional and behavioral levels. This work furthers our understanding of the link between MIA, poor nutritional habits and NDD, emphasising a potential role of the gut-brain axis.

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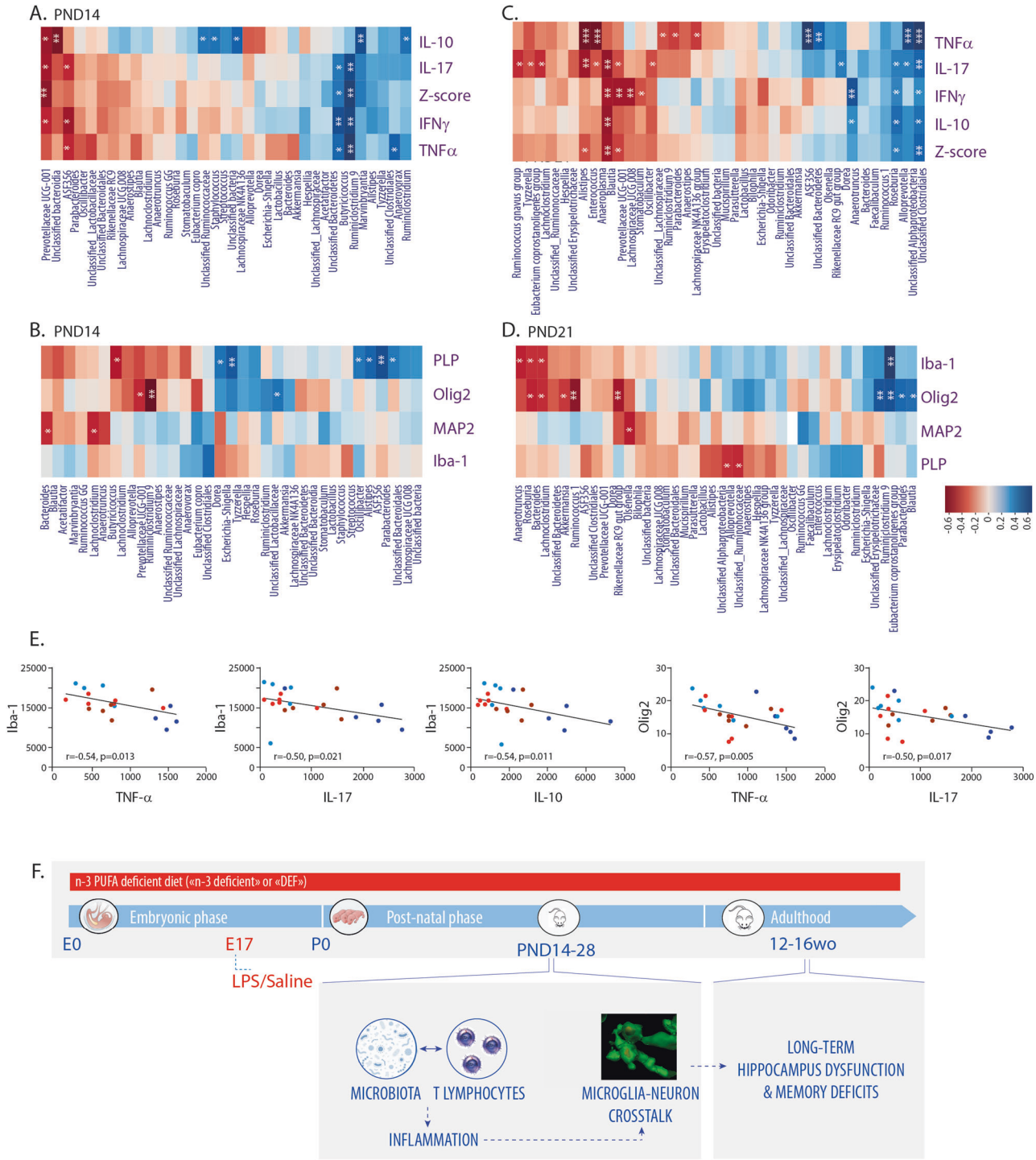

Fig. 5 Correlations between microbial modifications, gut inflammation, and neurobiological parameters. a Spearman's correlation matrix between gut immune cells reactivity (e.g cytokine release after T-cells stimulation) and bacterial genera in PND14 mice (*$p = 0.05$). **b** Spearman's correlation matrix between neurobiological measurements (PLP, Olig2, Iba-1 and MAP2) and bacterial genera in PND14 mice (*$p = 0.05$). **c** Spearman's correlation matrix between gut immune cells reactivity (e.g cytokine release after T-cells stimulation) and bacterial genera in PND21 mice (*$p = 0.05$). **d** Spearman's correlation matrix between neurobiological measurements (PLP, Olig2, Iba-1, and MAP2) and bacterial genera in PND21 mice (*$p = 0.05$). **e** Spearman's correlation between gut immune cells reactivity (e.g released cytokines after stimulation) and neurobiological parameters in PND21 mice (*$p = 0.05$). $N = 20–22$. Escherichia-Shig: Escherichia-Shigella; Eubacterium copro: Eubacterium coprostanoligenes group; Lachno NK4A136: Lachnospiraceae NK4A136 group; Lachno UCG-008: Lachnospiraceae UCG-008; Prevo UCG-001: Prevotellaceae UCG-001; Rikenellaceae RC9: Rikenellaceae RC9 gut group; Ruminococcus gg: Ruminococcus gnavus group. **f** Schematic summarizing the main findings. Exposure of n-3 PUFA deficient dams to MIA alters the gut microbiota composition and increases the inflammatory reactivity of the gut T-lymphocytes in the offspring during the post-natal period. This is correlated with an impairment in microglia-neuron crosstalk during this phase, with consequences on hippocampus function and memory abilities later in life.

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AUTHOR CONTRIBUTIONS

QL, FD, ARAAQ, IV, CL, ANB, JB, AA, AS, FC, LS, BM, TB, SG, JMC performed all animal experimentations. SG, CJ, and LB performed and analyzed lipid experiments on whole hippocampus. BM and TB performed physiological and behavioral measurements on neonates. GB performed and UR and PT oversaw bioinformatic analyses of transcriptomic data. CA performed correlation analyses. FG performed microbiota analyses. JMC and FC performed gut measurements. SL and AN equally supervised the entire project and wrote the manuscript. All authors proof-read the manuscript.

ADDITIONAL INFORMATION

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