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Supplementary Information for

Maternal viral infection causes global alterations in porcine fetal microglia

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Supplementary Information

S1.0 Materials and Methods

S1.1 Animals. Twenty-seven pregnant Large White/Landrace crossbred gilts were obtained from the PRRSV-free University of Illinois swine herd at gestational day (GD) 69 and maintained in disease containment chambers within the biomedical animal facility as previously described (1, 2). In brief, all gilts were littermate pairs and artificially inseminated with semen from the same boar (PIC 359 SS 6278, Birchwood Genetics, Inc., West Manchester, OH). One gilt from each littermate pair was inoculated intranasal with 5 mL of 1 x 10^5 50% tissue culture infectious dose ($TCID_{50}$) of live PRRSV (P-129-BV strain, obtained from Purdue University, West Lafayette, IN) at GD 76, while the alternate littermate received sterile DMEM. Six total replicates were completed, for a total of 13 gilts in the control group and 14 gilts in the PRRSV group. To confirm viral presence, oral fluids were collected from each gilt immediately prior to inoculation (GD 76) and at 7 days post inoculation (dpi; GD 83). Oral fluid collection consisted of allowing gilts to chew on cotton rope until saturated; extracted oral fluids were stored at -20°C until evaluation.

The study design is outlined in **Fig. 1A**. At GD 83 $\pm$ 1 d (7 dpi; peak maternal infection), half of the gilts were euthanized (control: n = 6; PRRSV: n = 7) for immediate collection of fetal tissues and microglia isolation. The other half (control: n = 7; PRRSV: n = 7) was euthanized at GD 97 $\pm$ 1 d (21 dpi; resolution of maternal symptoms). First, gilts were anesthetized through intramuscular administration of 0.04 mL/kg body weight telazol:ketamine:xylazine (TKX) drug cocktail (50 mg tiletamine plus 50 mg zolazepam reconstituted with 2.5 mL ketamine [100 g/L] and 2.5 mL xylazine [100 g/L]; Henry Schein Animal Health, Dublin, OH). Once anesthesia was confirmed, blood was collected from the marginal ear vein into blood collection tubes containing EDTA (for plasma) or clot activator (for serum). Blood tubes containing clot activator were allowed to clot at room temperature for $\geq$ 30 min; tubes containing EDTA were preserved on ice. Gilts were then euthanized through intravenous administration of a lethal dose (86 mg/kg) of sodium pentobarbital (Fatal Plus, Vortech Pharmaceutical, Dearborn, MI).

Upon confirmation of euthanasia, cesarean sections were performed as previously described (2). In brief, the uterus and both ovaries were removed and litter size was determined by gently palpating the uterus. Fetuses were given unique IDs, beginning closest to the ovary, for each left and right horn. Amniotic fluid was collected from each fetus by passing a needle into the amniotic cavity, then individual fetuses were extracted and categorized as viable or non-viable (3). Umbilical cord blood was collected from each fetus using vacutainer needles and blood collection tubes containing EDTA or clot activator. Blood tubes containing clot activator were allowed to clot at room temperature for $\geq$ 30 min; tubes containing EDTA were preserved on ice. All blood tubes were then centrifuged at 1,300 x g at 4°C for 15 min and serum and plasma were aliquoted and stored at -80°C.

The distal end of each umbilical cord was severed leaving minimal placental tissue attached. Fetal body weights (including exsanguinated umbilical cord) and brain weights were obtained. The placental tissue attached to the cord, and endometrial tissue immediately adjacent to the umbilical stump of each individual fetus, was collected and snap frozen on dry ice. The fetal brain was bisected, separating the left hemisphere from the right. The entire left hemisphere was immediately placed in 10% neutral buffered formalin and preserved for immunohistochemistry. Specific regions of interest (including the hypothalamus, hippocampus, amygdala, frontal cortex, and striatum) were dissected from the right hemisphere and snap frozen. The remaining brain tissue of the right hemisphere was minced with a scalpel blade to obtain a homogenous sample, then submerged in Hanks' balanced salt solution (without calcium and magnesium; HBSS; Mediatech, Inc., Manassas, VA) and placed on ice for microglial cell isolation. A subset of seven separate litters (eight representative fetuses from each, balanced for sex, weight, and uterine horn location) were reserved for microglia *in vitro* analyses; these animals were excluded from all other analyses. All animal husbandry and experimentation was in accordance with the National Research

Council *Guide for the Care and Use of Laboratory Animals* and was approved by the University of Illinois Institutional Animal Care and Use Committee.

S1.2 PRRSV and cytokine detection. Oral fluid and serum samples from gilts, and serum samples from fetal cord blood, were analyzed by the Veterinary Diagnostic Laboratory (University of Illinois, Urbana, IL) for presence of PRRSV through rRT-PCR. Inflammatory cytokines IL-6 and TNFα were measured in plasma from gilts and fetal cord blood, and in cell culture media, using porcine-specific DuoSet ELISA kits (R&D systems, Minneapolis, MN) as per manufacturer's instructions. Circulating IL-17a was measured in gilt plasma using a porcine-specific ELISA kit (Thermo Fisher Scientific, Waltham, MA) according to manufacturer's instructions.

S1.3 Microglial cell isolation. Fetal microglia were isolated using Miltenyi Biotech (San Diego, CA) cell separation techniques, based on positive selection with CD11b beads, as previously described (1), with few changes. Briefly, homogenized tissue from the right hemisphere was removed from HBSS and transferred to GentleMACS c-tubes containing triple the recommended volumes of Enzyme Mix 1 and 2 (NTDK[P]). Isolated CD11b+ microglia from each fetus were suspended in flow buffer (PBS with 1% BSA [Thermo Fisher Scientific, Waltham, MA] and 0.1% sodium azide [Sigma-Aldrich, St. Louis, MO]) and split in half: one half was used for flow cytometric analyses, and the other half was resuspended in 1 mL TRIzol™ Reagent (Invitrogen, Carlsbad, CA) for isolation of RNA. Samples from seven different litters were reserved for *in vitro* assays, and thus were instead suspended in 5 mL PEB (PBS with 2 mM EDTA and 0.5% BSA) and counted using a Z-Series Coulter Counter (Beckman Coulter Life Science, Indianapolis, IN).

S1.4 Flow cytometry. Flow cytometric procedures were carried out as previously described (1). Cells were incubated with the following antibodies: mouse anti-pig FITC-conjugated CD45 (AbD Serotec, Raleigh, NC), mouse anti-pig PE-conjugated MHCII (Antibodies Online, Atlanta, GA), and mouse anti-human APC-conjugated CD68 (BioLegend, San Diego, CA), then fixed using 10% neutral buffered formalin for 10 min. Cells were flowed through the FACS Aria II flow cytometer (BD Biosciences, San Jose, CA) and fluorescence intensity was compared against a massed unstained control sample.

S1.5 Stimulation of primary microglia cells with LPS and poly I:C. Isolated CD11b+ cells were suspended in standard cell culture medium (DMEM containing 100 U/mL penicillin/streptomycin, 10% FBS, and 1 μL/mL porcine rpGM-CSF [R&D Systems, Minneapolis, MN]) and plated in 12-well cell culture plates at ~4 x 10^6 cells per well. Cells were incubated with LPS (1 ng/mL media; *E. coli* 0127:B8, Sigma-Aldrich, St. Louis, MO), poly I:C (1 μg/mL media; Sigma-Aldrich, St. Louis, MO) or an equivalent volume of sterile D-PBS for 4 h, then supernatant was collected and stored at -80°C until analysis.

S1.6 Phagocytosis and Chemotaxis assays. Phagocytic and chemotactic activity of CD11b+ cells were assessed as previously described (4), using the Vybrant Phagocytosis Assay Kit (Life Technologies, Carlsbad, CA) and the Neuro Probe ChemoTx system (101-8; Neuro Probe, Inc., Gaithersburg, MD). Samples were plated in replicates of 4 for the phagocytosis assay, and replicates of 5 for the chemotaxis assay.

S1.7 RNA extraction, cDNA synthesis, and quantitative real-time-PCR. Total RNA from gilt and fetal tissues was extracted using the TRI Reagent® protocol (Sigma-Aldrich, St. Louis, MO). At least one representative sample per litter was analyzed for each tissue type. cDNA was synthesized from 1-4 μg of RNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Grand Island, NY). Quantitative real-time PCR was performed using the Applied Biosystems TaqMan Gene Expression Assay protocol (Thermo Fisher Scientific, Waltham, MA); samples were assayed in duplicate. Housekeeping gene

ribosomal protein L19 (RPL19) was used for calculating relative fold change of target genes (listed in **Table S7**) using the delta-delta CT method.

RNA was isolated from the hypothalamus of 3-4 representative fetuses from each litter and cDNA was generated. RT-PCR results for IL-1RA at 7 dpi were used to run a power analysis (using 'proc power' for a one-way ANOVA in Statistical Analysis Software 9.4; SAS Institute, Cary, NC) based on a standard deviation of 0.2 and 90% power, which revealed that 7 fetuses from each treatment group were needed. Thus, two-three representative fetuses from each litter were selected for any future analyses. Samples were excluded if RNA yield was below 1 μg, resulting in a total of 9-11 per treatment group at both 7 and 21 dpi.

S1.8 High-throughput qPCR using the Fluidigm amplification system. cDNA from fetal microglia and amygdala tissue was submitted to the W.M. Keck Center for Comparative and Functional Genomics (University of Illinois, Urbana, IL) for quantitative PCR analysis using the Biomark HD Fluidigm® high-throughput amplification system. Forty-eight genes of interest (selected from (5); TaqMan assays; **Table S3**) were assessed on the 96 x 96 platform, and all samples were run in duplicate. Data were analyzed using the Biomark & EP1 Real-Time PCR Analysis Software with quality threshold set at 0.65, baseline correction set at 'Linear (Derivative)', and Ct threshold method of 'Auto (Global)'. Delta delta Ct calculations were used to obtain fold change of target genes compared to housekeeping control RPL19.

S1.9 Iba1 immunohistochemistry. A stereotaxic atlas for the Large White pig brain (6) was used to calculate the correct orientation and slicing of the left hemisphere to obtain coronal slices containing the hippocampus and amygdala. Coronal sections (~5 mm thick) were placed into tissue cassettes and post-fixed for 24-48 h in zinc formalin (Fisher Scientific, Hampton, NH). Paraffin embedding, slicing (10 μm sections), and slide mounting was done by the Veterinary Diagnostic Laboratory (University of Illinois, Urbana, IL). Anti-Iba1 rabbit primary antibody (Wako, Richmond, VA) was used at 1:500 dilution and goat anti-rabbit IgG secondary antibody (Jackson ImmunoResearch Inc., West Grove, PA) at 1:5000 dilution. Signal was amplified using Vector ABC kit PK4000 (Vector Laboratories, Burlingame, CA) and color was developed using 3,3'-Diaminobenzidine tetrahydrochloride (DAB) tablets (Sigma Aldrich, St. Louis, MO). Stained slides were counterstained with hematoxylin and cover-slipped with DPX Mounting Medium (Sigma Aldrich, St. Louis, MO). A total of 10-11 individual fetuses per treatment group (two-three per litter), per time point, were stained.

S1.10 Microglia counting and morphology. Digital images of Iba1-stained slides were obtained at 40X magnification using a NanoZoomer slide scanner (Hamamatsu Photonics, Hamamatsu, Japan). Images were analyzed using the NDP.view2 software (Hamamatsu Photonics, Hamamatsu, Japan) by trained observers blinded to treatment. Regions of interest (the amygdala and the dentate gyrus and hilar region of the hippocampus) were located using the stereotaxic pig brain atlas (6) and traced using the "Freehand Region" annotation tool. Individual Iba1+ cells were marked and totaled for each region of interest, and the number of cells per mm^2 was calculated.

The morphology of Iba1+ cells were then categorized based on four defined phenotypes: cells with 'Thin, Ramified' processes (or quiescent microglia); cells with 'Long, Thick' processes; cells with short 'Stout' processes; or 'Amoeboid' activated cells, which have enlarged somas and few to zero processes (representative images are presented in **Fig. 6A**). All cells within the dentate gyrus/hilar region were assessed, while only cells within a 1 mm^2 box ('Mitotic Density Box' annotation tool) placed in the center of the amygdala were assessed. The total number of cells within each category was summed and total Iba1+ cells per mm^2 per morphological category was calculated. Three slides per fetus were analyzed for the dentate gyrus/hilar region (due to the dense orientation of hematoxylin+ cells), and then averaged.

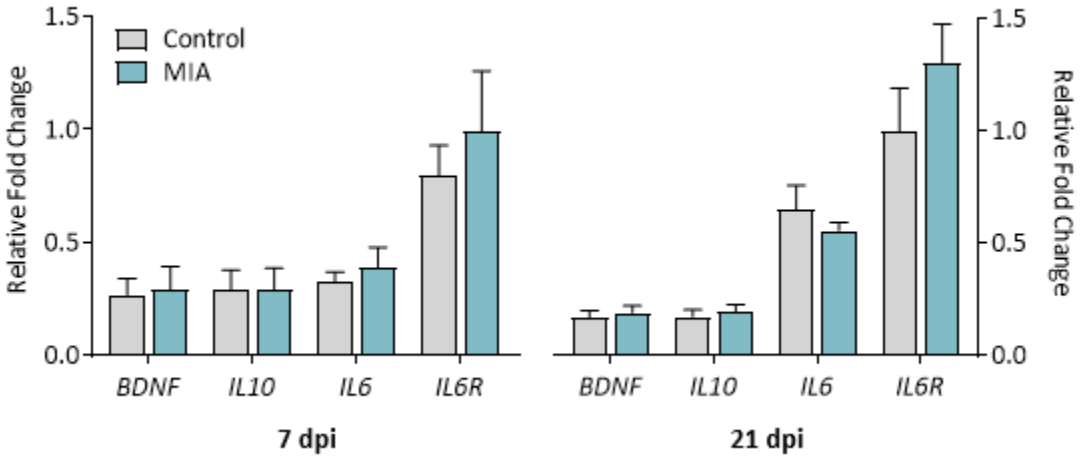
Microglia soma characterization was then carried out on Iba1+ cells within these same regions. An average of 92 cells were quantified within the dentate gyrus/hilar region of the hippocampus, while an average of 103 cells were quantified within the 1 mm^2 box in the center of the amygdala. Soma length and width was measured using the 'Linear Measure' annotation tool; length was defined as the distance between the farthest points of the soma, and width was defined as the widest distance perpendicular to soma length (as previously described (4)). Soma area was estimated by using the length and width measurements divided by 2 to create two radii, and by using the area calculation for an ellipse (radius A*radius B*π).

Slide exclusion criteria included, 1) slide or tissue damage during staining; 2) air bubbles within the DPX mounting media that obscured the region of interest; and 3) portions of the region of interest were out of focus.

S1.11 Statistics. GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA) and Statistical Analysis Software 9.4 (SAS Institute, Cary, NC) were used for all statistical analyses, with an α of 0.05 and trends reported at $p \leq 0.10$. 'Proc mixed' or 'proc glm' procedures were used in SAS 9.4 for almost all analyses; a 'repeated' statement was included in the model for gilt body temperature and food intake. For any significant interactions, the 'lsmeans' statement was used with a Bonferroni adjustment to assess means separation. For tests with only one variable (i.e. maternal infection or gestational day), unpaired t tests were performed using GraphPad 8; if variances were unequal, a Welch's correction was used. All fetal groups were balanced for body weight and left and right uterine horn placement; all fetal groups were balanced for sex when possible. Main effects of sex or MIA*sex interactions are reported within the main text for sufficiently powered 2-way ANOVA tests. For analyses that were underpowered for comparisons across sex (gene expression and Iba1 immunohistochemistry), secondary statistical tests were performed with sex included as a biological variable, and these data are presented as supplementary figures or tables, and are not included in the main text.

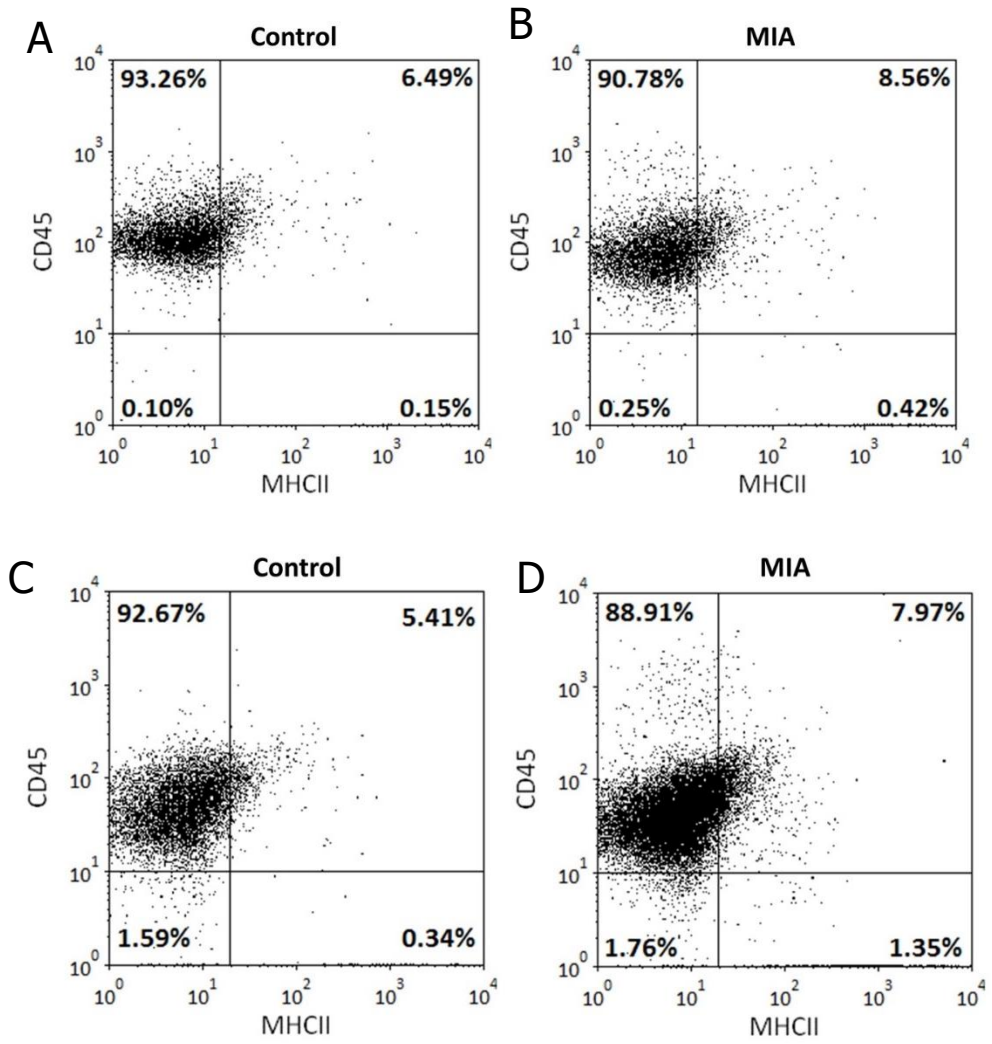
S2.0 Supplemental Figures and Tables:

Supplemental Figure S1



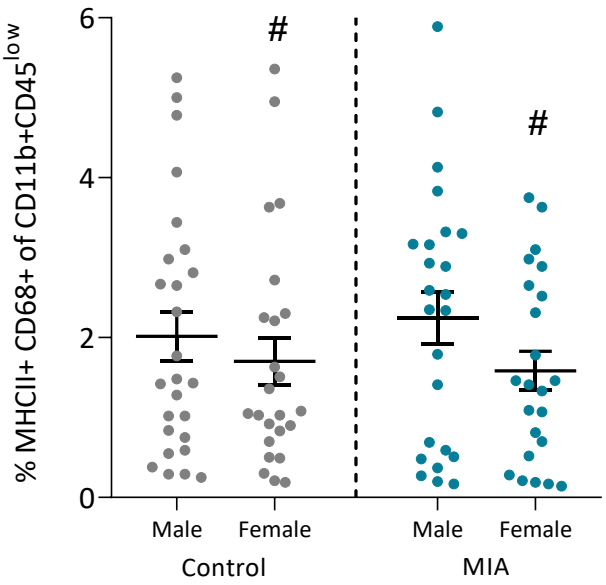
Supp. Fig. S1. Placental gene expression at 7 and 21 dpi. Placental tissue was collected near the distal end of the umbilical cord. There were no significant differences between maternal treatment groups using 1-way ANOVA testing; 2-way ANOVA testing (MIA*sex) revealed an interaction at 21 dpi for expression of *BDNF* and *IL10* ($p < 0.05$), such that control females appeared to express *BDNF* and *IL10* at higher levels compared to control males and MIA females, but not MIA males (Tukey post hoc testing was not significant). Error bars are ± SEM; n = 6-11 fetuses per treatment group, per time point; dpi = days post inoculation; MIA = maternal immune activation.

Supplemental Figure S2



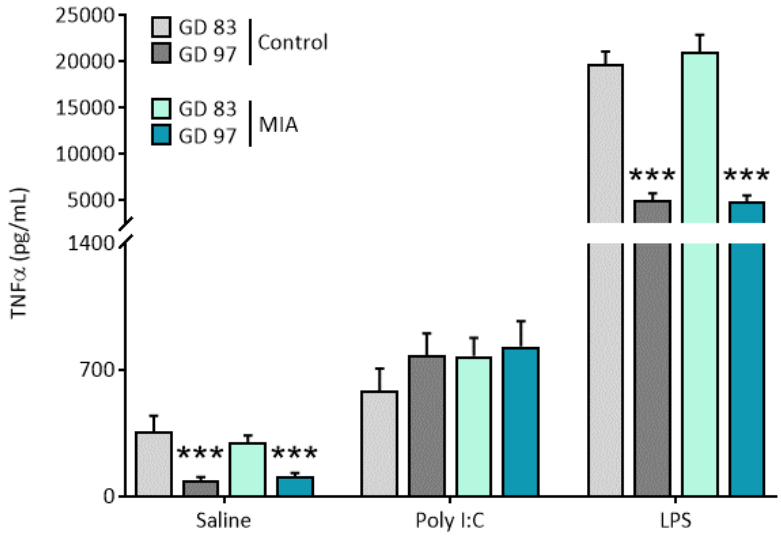
Supp. Fig. S2. Representative flow cytometry scatter plots of microglia. Percent of CD11b+ cells that express CD45 and MHCII. CD11b+ cells isolated from fetal brains of control and MIA piglets at 7 dpi (**A, B**) and 21 dpi (**C, D**); dpi = days post inoculation; MIA = maternal immune activation.

Supplemental Figure S3.



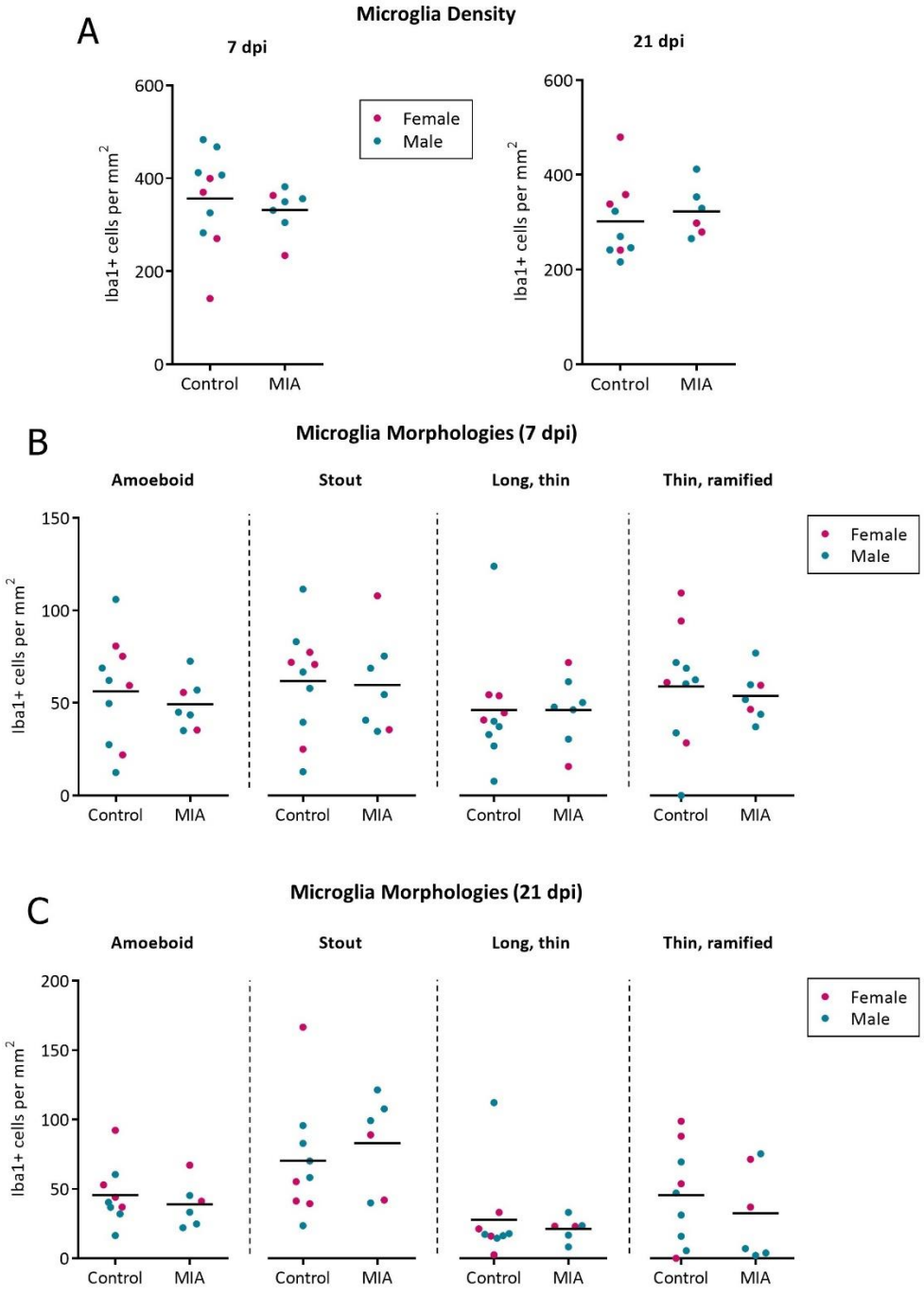
Supp. Fig. S3. Sex differences in microglia MHCII and CD68 co-expression at 21 dpi. Percent of CD11b+ $CD45^{low}$ microglia isolated from fetal brains co-expressing antigen-presenting and phagocytic markers MHCII and CD68. Male fetuses tended ($p = 0.10$) to have higher expression of MHCII and CD68 compared to females, regardless of maternal treatment. Each dot represents an individual animal; error bars are ± SEM; n = 23-26 fetuses per sex per treatment group; # = main effect of sex, $p \leq 0.10$; dpi = days post inoculation; MIA = maternal immune activation.

Supplemental Figure S4.



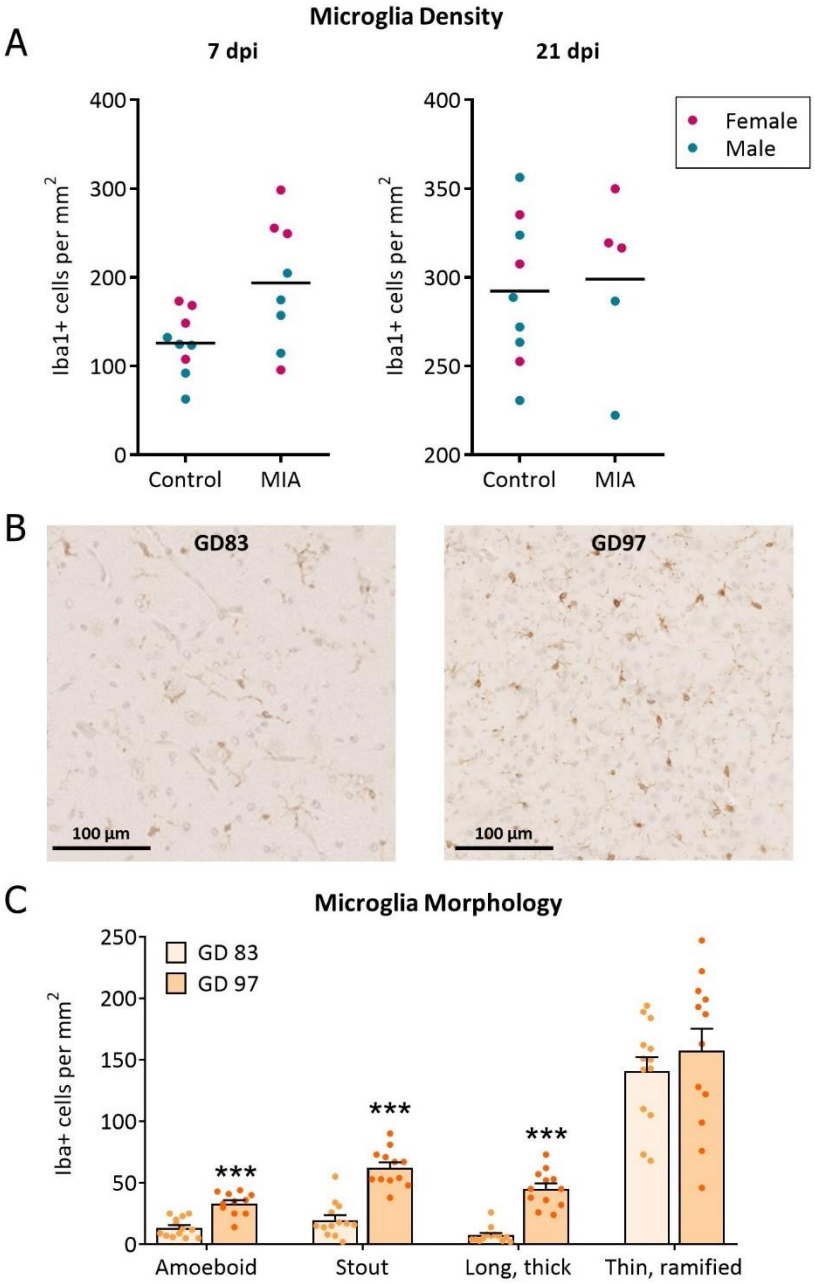
Supp. Fig. S4. *In vitro* stimulation of primary fetal microglia isolated from control and MIA piglets. Maternal infection did not impact production of pro-inflammatory cytokine TNFα by primary microglia 4 h post-stimulation with saline, poly I:C (1 μg/mL), or LPS (1 ng/mL). However, TNFα protein concentration in culture media was reduced in cells isolated at GD 97 (21 dpi) compared to GD 83 (7 dpi; *in vitro* treatment x gestational day, $p < 0.0001$). Four million primary cells per piglet, per treatment; error bars are ± SEM; n = 7-16 fetuses per group. *** = individual ANOVA for each *in vitro* treatment group, main effect of gestational day, $p < 0.0001$. MIA = maternal immune activation; GD = gestational day.

Supplemental Figure S5



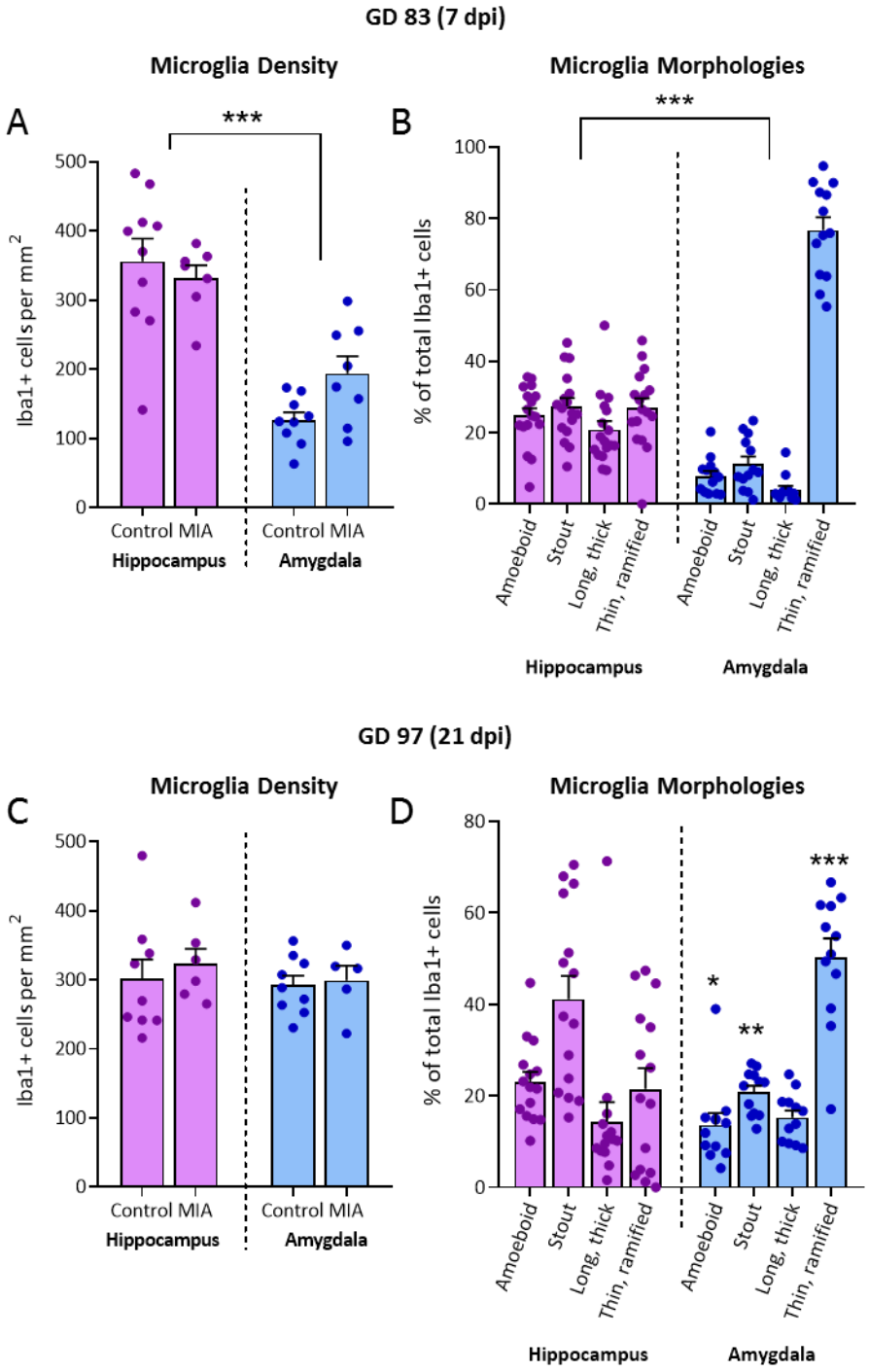
Supp. Fig. S5. Microglia density and morphologies in the hippocampus. Maternal infection had few impacts on total number and morphology of fetal microglia in the dentate gyrus and hilar regions of the fetal hippocampus. 2-way ANOVA testing for these data, which included sex as a variable, revealed (**A**) at 7 dpi, males tended to have more total microglia compared to females ($p \leq 0.10$), regardless of MIA treatment. At 21 dpi, there tended to be an interaction of MIA and sex on total microglia number ($p = 0.07$). Microglia morphologies at 7 dpi (**B**) did not differ between sex or treatment groups. At 21 dpi (**C**), females tended to have a higher number of amoeboid microglia compared to males, though there was no effect of maternal treatment. Horizontal line represents the group mean; n = 6-10 individual fetuses per treatment group; MIA = maternal immune activation.

Supplemental Figure S6



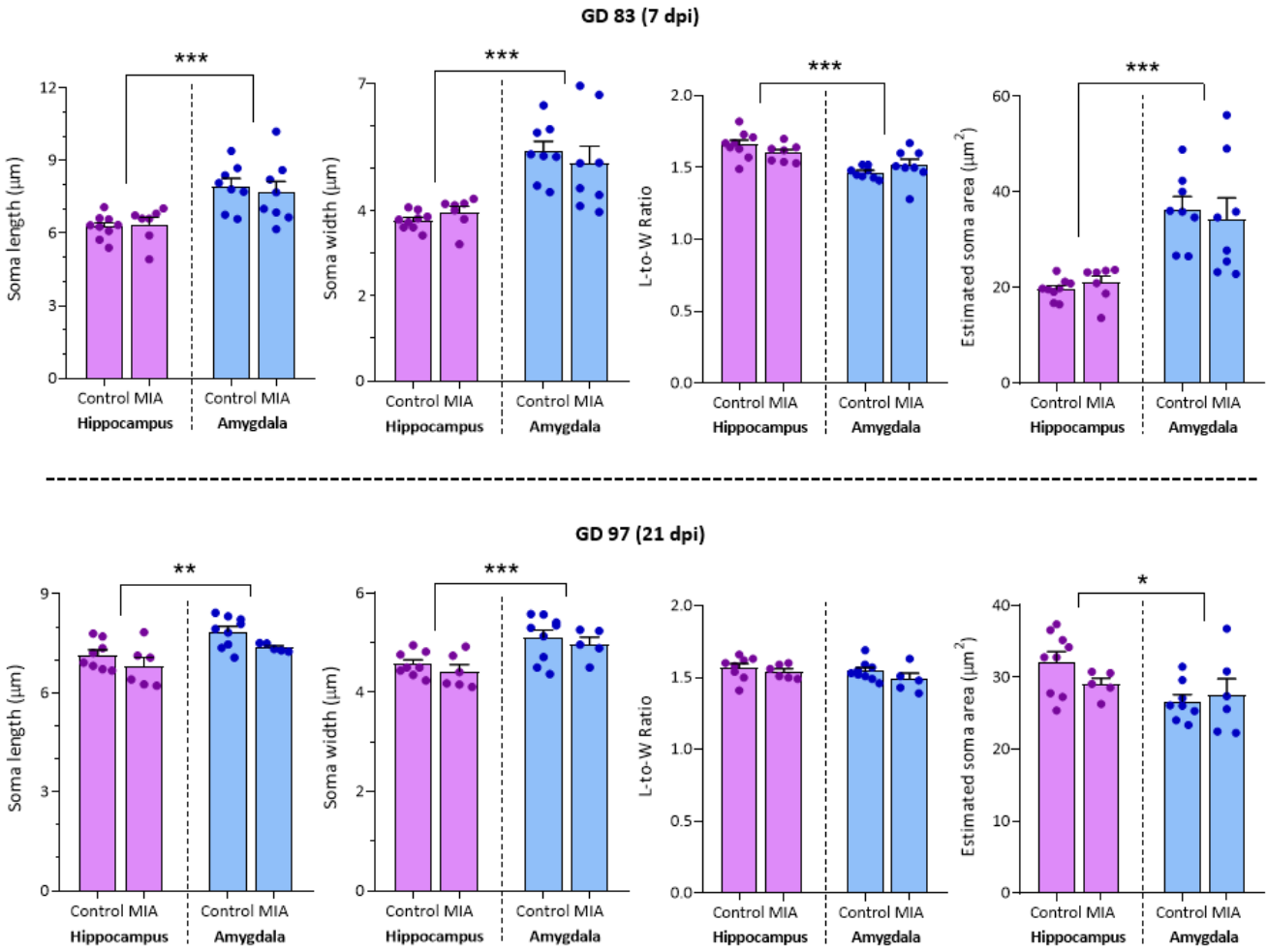
Supp. Fig. S6. Microglia density and morphologies in the fetal amygdala. 2-way ANOVA testing for density of Iba1+ cells, which included sex as a variable, revealed (**A**) female fetuses tended to have more microglia in the amygdala compared to males at both 7 dpi ($p = 0.056$) and 21 dpi ($p = 0.10$); n = 5-9 individual fetuses per treatment group; horizontal line represents the group mean. (**B**) Representative images of microglial densities and morphologies at 40X magnification at GD 83 and GD 97; scale bar = 100 µm. (**C**) There was an increase in the number of Iba1+ cells classified as amoeboid, having stout processes, or having long, thick processes at GD 97 compared to GD 83 ($p < 0.001$). The number of microglia classified as having thin, ramified processes did not differ across time points. Error bars are ± SEM; n = 12-13 per gestational day; *** = main effect of gestational age, $p < 0.001$. MIA = maternal immune activation; GD = gestational day.

Supplemental Figure S7



Supp. Fig. S7. Differences in microglia density and morphology based on brain region and gestational age. Density of Iba1+ cells was reduced in the amygdala compared to the hippocampus at 7 dpi (**A**; $p < 0.0001$), though microglia density in the amygdala increased between GD 83 and GD 97, bringing total density up to comparable levels with the hippocampus (**C**) at 21 dpi; n = 14-18 fetuses per brain region. The percent of Iba1+ microglia categorized into each of four morphologies differed across all categories at 7 dpi (**B**), and all but one category at 21 dpi (**D**). N = 12 – 17 fetuses per brain region; main effect of brain region, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Error bars are ± SEM.

Supplemental Figure S8



Supp. Fig. S8. **Differences in cell soma measurements based on brain region and gestational age.**
Top panel: Microglia soma measurements from GD 83 fetuses. Soma length, width, and estimated area was increased in the fetal amygdala compared to the dentate gyrus/hilar region of the hippocampus. This resulted in an overall decrease in length-to-width ratio of microglia somas in the amygdala. **Lower panel**: Measurements from GD 97 fetuses. Soma length, width, and estimated area was increased in the fetal amygdala compared to the dentate gyrus/hilar region of the hippocampus, but length-to-width ratio did not differ. Error bars are ± SEM; n = 14 – 16 fetuses per brain region; main effect of brain region, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; GD = gestational day; MIA = maternal immune activation.

Supplemental Table S1. Fetal body and brain weight (by treatment group, sex, and time point).

Treatment Group (Sex)	7 dpi			21 dpi		
	Body Wt. (g)	**Brain Wt. (g)**	**N**	**Body Wt. (g)**	**Brain Wt. (g)**	**N**
Control (male)	534.9 ± 24.0	14.99 ± 0.22	21	862.5 ± 22.6	26.07 ± 0.38	26
MIA (male)	556.3 ± 20.7	15.62 ± 0.22	18	813.7 ± 34.1	**24.28 ± 0.34*** **	24
Control (female)	527.3 ± 15.9	14.96 ± 0.24	24	846.3 ± 31.5	24.33 ± 0.38	24
MIA (female)	522.3 ± 23.0	14.96 ± 0.27	21	831.6 ± 43.7	**22.83 ± 0.39*** **	23

Data are mean ± SEM. Significant main effect of litter size ($p < 0.0001$) on both parameters. No differences between treatment groups at 7 dpi for body or brain weight; significant main effect of maternal PRRSV at 21 dpi on brain weight ($p < 0.0001$; no maternal treatment*litter size interaction), but not body weight. Significant main effect of sex at 21 dpi on brain weight ($p < 0.0001$; no maternal treatment*sex interaction). Brain-to-body weight ratio did not differ across maternal treatment or sex for either time point. N = 5 litters per treatment group for each time point (20 litters total); n/sex/group presented within the table; *** = main effect of maternal treatment $p < 0.0001$ (bold); dpi = days post infection; MIA = maternal immune activation.

Supplemental Table S2. Average litter characteristics.

Treatment Group	Litter Size	Viable Piglets	Non-viable Piglets
Control (n = 10)	11.1 ± 1.1	9.5 ± 1.3	1.6 ± 0.6
PRRSV (n = 10)	12.4 ± 1.1	8.7 ± 1.4	2.3 ± 0.8

Data are means ± SEM. No significant differences between treatment groups. However, the current study was not designed to detect significant litter differences due to maternal PRRSV infection and thus is underpowered for those analyses. These data are merely meant to summarize the average characteristics of the litters used in the study; PRRSV = porcine reproductive and respiratory syndrome virus.

Supplemental Table S3. Fluidigm rt-PCR primer information.

Gene	*Accession*	*Category*	*Applied Biosystems Assay ID*[a]
BDNF	NM_214259	Development	Ss03822335_s1
C1QA	NM_001003924	Development	Ss03378489_u1
C5	NM_001001646	Development	Ss03391586_m1
CCR5	NM_001001618	Sensome	Ss03378121_u1
CCRL2	NM_001001617	Sensome	Ss03378109_u1
CD14	NM_001097445	Sensome	Ss03818718_s1
CD180	NM_214357	Sensome	Ss03384320_s1
CD4	NM_001001908	Sensome	Ss03391676_m1
CD40	NM_214194	Sensome	Ss03394337_m1
CD74	NM_213774	Sensome	Ss03381367_u1
CD86	NM_214222	Sensome	Ss03394401_m1
CDK1	NM_001159304	Development	Ss03372912_g1
CRYBB1	NM_001078681	Development	Ss03388726_m1
CSF1	AJ583705	Development/Sensome	Ss03373560_g1
CXCL16	NM_213811	Sensome	Ss03381587_u1
CXCL2	NM_001001861	Immune	Ss03378360_u1
CXCR2	AK230995	Development	Ss03375929_g1
DNMT1	NM_001032355	Development	Ss03392016_m1
DNMT3A	NM_001097437	Development	Ss03385484_u1
EGR-1	AJ238156	Development	Ss03373483_s1
FCER1G	NM_001001265	Sensome	Ss03391475_m1
FCGR2B	NM_001033013	Sensome	Ss03392060_m1
FCGR3	NM_214391	Sensome	Ss03384597_u1
IFIT3	NM_001204395	Development	Ss04248506_s1
IFNG	NM_213948	Immune	Ss03391054_m1
IFNGR1	NM_001177907	Sensome	Ss04246620_m1
IL10	NM_214041	Immune	Ss03382372_u1
IL1B	NM_214055	Immune	Ss03393804_m1
IL17A	NM_001005729	Immune	Ss03391803_m1
IL6	NM_214399	Immune	Ss03384604_u1
IL8	NM_213867	Immune	Ss03392437_m1
ITGAM	JF709973	Immune/Sensome	Ss03374588_m1
ITGB5	NM_001246669	Sensome	Ss04322936_m1
LY86	NM_001097415	Sensome	Ss03388794_m1
NOS2	NM_001143690	Immune	Ss03374608_u1
P2RY12	NM_001173518	Sensome	Ss03373817_s1
P2RY6	NM_001244296	Sensome	Ss03376684_u1
RELA	NM_001114281	Immune	Ss03253758_m1
RPL19	AF435591	Housekeeping	ss03375624_g1
SELPLG	NM_001105307	Sensome	Ss04328834_s1
SLC2A5	EU012359	Sensome	Ss03377332_u1
TGFB1	NM_214015	Development	Ss03382325_u1
TGFBR1	NM_001038639	Sensome/Development	Ss03392139_m1
TLR2	NM_213761	Sensome	Ss03381278_u1
TNF	NM_214022	Immune	Ss03391318_g1
TNFRSF1B	NM_001097441	Sensome	Ss03385518_u1
TPI1	NM_001037151	Development	Ss03379689_u1
TYROBP	NM_214202	Sensome	Ss03394361_m1

Supplemental Table S4. Differences of Least Squares Means for the interaction between MIA and sex on fetal microglia gene expression.

Tissue	DPI	Gene	MIA*Sex Comparison		Bonferroni adjusted p-value
Microglia	21	*C1QA*	Control F	Control M	**0.0008 *** **
			Control F	MIA F	**0.01 ***
			Control F	MIA M	**0.015 ***
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*C5*	Control F	Control M	**0.0004 *** **
			Control F	MIA F	**0.003 ** **
			Control F	MIA M	**0.006 ** **
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*CDK1*	Control F	Control M	**0.009 ** **
			Control F	MIA F	**0.044 ***
			Control F	MIA M	*0.06 #*
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*CSF1*	Control F	Control M	**0.021 ***
			Control F	MIA F	**0.018 ***
			Control F	MIA M	**0.029 ***
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*DNMT1*	Control F	Control M	**0.0003 *** **
			Control F	MIA F	**0.004 ** **
			Control F	MIA M	**0.004 ** **
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*DNMT3A*	Control F	Control M	**0.0002 *** **
			Control F	MIA F	**0.003 ** **
			Control F	MIA M	**0.002 ** **
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*TGFB1*	Control F	Control M	**< 0.0001 *** **
			Control F	MIA F	**0.001 ** **
			Control F	MIA M	**0.001 ** **
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1

Supplemental Table S4 (continued).

Tissue	DPI	Gene	MIA*Sex Comparison		Bonferroni adjusted p-value
Microglia	21	*TGFBR1*	Control F	Control M	**0.007 ****
			Control F	MIA F	**0.03 ***
			Control F	MIA M	*0.07 #*
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*TPI1*	Control F	Control M	**0.0002 *****
			Control F	MIA F	**0.0005 *****
			Control F	MIA M	**0.001 ****
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*CCR5*	Control F	Control M	**0.0001 *****
			Control F	MIA F	**0.01 ***
			Control F	MIA M	**0.002 ****
			Control M	MIA F	0.62
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*CD14*	Control F	Control M	**0.0001 *****
			Control F	MIA F	**0.002 ****
			Control F	MIA M	**0.002 ****
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*CD180*	Control F	Control M	**0.007 ****
			Control F	MIA F	0.127
			Control F	MIA M	0.104
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*CD4*	Control F	Control M	**0.01 ***
			Control F	MIA F	**0.004 ****
			Control F	MIA M	**0.002 ****
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*CD40*	Control F	Control M	**0.0004 *****
			Control F	MIA F	**0.005 ****
			Control F	MIA M	**0.009 ****
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1

Supplemental Table S4 (continued).

Tissue	DPI	Gene	MIA*Sex Comparison		Bonferroni adjusted p-value
Microglia	21	*CD74*	Control F	Control M	**0.0002** ***
			Control F	MIA F	**0.006** **
			Control F	MIA M	**0.003** **
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*CD86*	Control F	Control M	**0.0007** ***
			Control F	MIA F	*0.07 #*
			Control F	MIA M	**0.02** *
			Control M	MIA F	0.53
			Control M	MIA M	0.92
			MIA F	MIA M	1
Microglia	21	*CXCL16*	Control F	Control M	**0.003** **
			Control F	MIA F	*0.09 #*
			Control F	MIA M	**0.02** *
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*FCGR2B*	Control F	Control M	**0.001** ***
			Control F	MIA F	**0.002** **
			Control F	MIA M	**0.003** **
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*FCGR3*	Control F	Control M	**0.0002** ***
			Control F	MIA F	**0.003** **
			Control F	MIA M	**0.0006** ***
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*IFNGR1*	Control F	Control M	**0.0009** ***
			Control F	MIA F	**0.02** *
			Control F	MIA M	**0.02** *
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*ITGAM*	Control F	Control M	**0.0006** ***
			Control F	MIA F	**0.006** **
			Control F	MIA M	**0.008** **
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1

Supplemental Table S4 (continued).

Tissue	DPI	Gene	MIA*Sex Comparison		Bonferroni adjusted p-value
Microglia	21	*ITGB5*	Control F	Control M	**0.001 ****
			Control F	MIA F	**0.006 ****
			Control F	MIA M	**0.02 ***
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*LY86*	Control F	Control M	**0.001 ****
			Control F	MIA F	0.24
			Control F	MIA M	0.11
			Control M	MIA F	0.24
			Control M	MIA M	0.35
			MIA F	MIA M	1
Microglia	21	*P2RY6*	Control F	Control M	**0.002 ****
			Control F	MIA F	**0.03 ***
			Control F	MIA M	**0.03 ***
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*P2Y12R*	Control F	Control M	**0.0004 *****
			Control F	MIA F	**0.009 ****
			Control F	MIA M	**0.003 ****
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*SELPLG*	Control F	Control M	**0.001 ****
			Control F	MIA F	**0.004 ****
			Control F	MIA M	**0.01 ***
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*SLC2A5*	Control F	Control M	**0.003 ****
			Control F	MIA F	**0.01 ***
			Control F	MIA M	**0.02 ***
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*TLR2*	Control F	Control M	**0.0007 *****
			Control F	MIA F	**0.01 ***
			Control F	MIA M	**0.008 ****
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1

Supplemental Table S4 (continued).

Tissue	DPI	Gene	MIA*Sex Comparison		Bonferroni adjusted p-value
Microglia	21	*TNFRSF1B*	Control F	Control M	**0.0008 *** **
			Control F	MIA F	**0.04 ***
			Control F	MIA M	**0.02 ***
			Control M	MIA F	0.72
			Control M	MIA M	0.92
			MIA F	MIA M	1
Microglia	21	*TYROBP*	Control F	Control M	**0.0002 *** **
			Control F	MIA F	**0.001 ** **
			Control F	MIA M	**0.001 ** **
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*IL10*	Control F	Control M	**0.003 ** **
			Control F	MIA F	0.18
			Control F	MIA M	**0.02 ***
			Control M	MIA F	0.73
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*IL1B*	Control F	Control M	**0.002 ** **
			Control F	MIA F	**0.006 ** **
			Control F	MIA M	**0.009 ** **
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*NOS2*	Control F	Control M	**0.007 ** **
			Control F	MIA F	*0.05 #*
			Control F	MIA M	**0.02 ***
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1
Microglia	21	*RELA*	Control F	Control M	**0.0002 *** **
			Control F	MIA F	**0.002 ** **
			Control F	MIA M	**0.003 ** **
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1

Least squares means separation for each MIA*sex comparison, expressed as Bonferroni adjusted p-values. N = 10-15/sex/maternal treatment group. Comparisons are for gene expression from primary microglia at 21 d post-inoculation; significant adjusted p-values are in bold; trends are italicized; *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$; # = $p \leq 0.10$. DPI = days post inoculation; F = female; M = male; MIA = maternal immune activation.

Supplemental Table S5. Gene expression in fetal microglia (7 and 21 d post-inoculation).

Tissue	Days post-inoculation	Gene	Treatment	Mean ± SEM	N	P-value
Microglia	7	*CCL2*	Control	1.00 ± 0.14	9	0.60
			MIA	1.12 ± 0.18	8	
		CD200R	Control	1.00 ± 0.17	9	0.60
			MIA	0.86 ± 0.21	8	
		IL6R	Control	1.00 ± 0.15	9	0.76
			MIA	1.08 ± 0.21	8	
		SLA-DRA (MHCII)	Control	1.00 ± 0.09	9	0.86
			MIA	1.04 ± 0.21	8	
		NFKB	Control	0.93 ± 0.08	8	0.81
			MIA	0.98 ± 0.18	8	
		STAT3	Control	1.00 ± 0.13	9	0.69
			MIA	0.91 ± 0.18	8	
Microglia	21	*CCL2*	Control	1.00 ± 0.12	10	0.23
			MIA	1.32 ± 0.22	10	
		CD200R	Control	0.68 ± 0.11	9	0.20
			MIA	0.50 ± 0.07	8	
		IL6R	Control	1.03 ± 0.02	7	**0.02***
			MIA	1.21 ± 0.06	8	
		SLA-DRA (MHCII)	Control	0.96 ± 0.08	8	0.81
			MIA	1.01 ± 0.19	10	
		NFKB	Control	1.00 ± 0.04	10	0.23
			MIA	0.92 ± 0.05	7	
		STAT3	Control	1.00 ± 0.04	10	0.28
			MIA	0.90 ± 0.08	10	

Values are expressed as relative fold change (mean ± SEM). 2-way ANOVA testing for these data, which included sex as a variable, revealed a main effect of MIA ($p = 0.001$) and a main effect of sex ($p = 0.009$), but no MIA-by-sex interaction for *IL6R* expression at 21 dpi, with females displaying a higher expression level compared to males. * = $p < 0.05$ (bold); MIA = maternal immune activation.

Supplemental Table S6. Quantitative rt-PCR primer information.

Gene	Accession	Category	Assay ID[a]
CCL2	NM_214214	Immune	Ss03394377_m1
CD200R[b]	Custom	Immune	custom
CX3CL1	DQ991100	Immune	Ss03377157_u1
CXCR3	AJ851240	Immune	Ss03375858_u1
IFNG	NM_213948	Immune	Ss03391054_m1
IL10	NM_214041	Immune	Ss03382372_u1
IL1B	NM_214055	Immune	Ss03393804_m1
IL1RA	NM_214262	Immune	Ss03383715_u1
IL6	NM_214399	Immune	Ss03384604_u1
IL6R	NM_214403	Immune	Ss03394904_g1
SLA-DRA (MHCII)	NM_001113706	Immune	ss03389942_m1
NFKB	NM_001048232	Immune	Ss03388575_m1
RPL19	AF435591	Housekeeping	ss03375624_g1
STAT3	NM_001044580	Immune	Ss03388426_m1
TNF	NM_214022	Immune	Ss03391318_g1

[a]Applied Biosystems TaqMan Gene Expression assay identification number.
[b] CD200R custom probe: forward primer, TGTTCCAAGTTACTAATCAGGCTGAA; reverse primer, AGCCCATTAGCAACATGATACTCTTT; probe, ACATAGAATTGAAGGAAGGG

Supplemental Table S7. Differences of Least Squares Means for the interaction between MIA and sex on fetal amygdala gene expression.

Tissue	DPI	Gene	MIA*Sex Comparison		Bonferroni adjusted p-value
Amygdala	7	*IFNGR1*	Control F	Control M	**0.03 ***
			Control F	MIA F	**0.03 ***
			Control F	MIA M	**0.01 ***
			Control M	MIA F	1
			Control M	MIA M	1
			MIA F	MIA M	1

Least squares means separation for each MIA*sex comparison, expressed as Bonferroni adjusted p-values. N = 11-14/sex/maternal treatment group. Comparisons are for gene expression from amygdala tissue at 7 d post-inoculation; significant adjusted p-values are in bold; * = $p < 0.05$. DPI = days post inoculation; F = female; M = male; MIA = maternal immune activation.

Supplemental Table S8. Gene expression in fetal hippocampal and hypothalamic tissue (7 d post-inoculation).

Tissue	Days post-inoculation	Gene	Treatment	Mean ± SEM	N	P-value
Hippocampus	7	*IFNG*	Control	0.72 ± 0.20	9	0.15
			MIA	0.38 ± 0.11	9	
		IL10	Control	0.83 ± 0.13	9	0.65
			MIA	0.75 ± 0.10	10	
		IL1B	Control	1.00 ± 0.29	8	0.44
			MIA	1.25 ± 0.12	9	
		IL6	Control	0.88 ± 0.09	9	0.48
			MIA	0.80 ± 0.07	10	
		STAT3	Control	0.88 ± 0.08	9	0.45
			MIA	0.79 ± 0.10	10	
		TNF	Control	1.00 ± 0.13	9	0.23
			MIA	1.21 ± 0.11	10	
Hypothalamus	7	*IFNG*	Control	0.92 ± 0.09	17	0.72
			MIA	0.98 ± 0.13	15	
		IL10	Control	0.91 ± 0.06	18	0.69
			MIA	0.95 ± 0.09	19	
		IL1B	Control	0.57 ± 0.09	18	0.36
			MIA	0.69 ± 0.09	17	
		IL6	Control	1.03 ± 0.06	19	**0.007****
			MIA	0.78 ± 0.06	18	
		STAT3	Control	0.95 ± 0.04	19	0.41
			MIA	0.89 ± 0.06	19	
		TNF	Control	0.81 ± 0.10	19	0.35
			MIA	0.94 ± 0.09	19	
		IL1RA	Control	0.97 ± 0.14	19	**0.02***
			MIA	0.58 ± 0.07	13	

Values are expressed as relative fold change (mean ± SEM). 2-way ANOVA testing for these data, which included sex as a variable, revealed no main effects of sex or MIA-by-sex interaction. ** = $p < 0.01$, * = $p < 0.05$ (bold); MIA = maternal immune activation.

Supplemental Table S9. Gene expression in fetal hippocampal and hypothalamic tissue (21 d post-inoculation).

Tissue	Days post-inoculation	Gene	Treatment	Mean ± SEM	N	P-value
Hippocampus	21	CX3CL1	Control	1.00 ± 0.21	10	0.62
			MIA	0.85 ± 0.22	9	
		IFNG	Control	0.56 ± 0.19	7	0.82
			MIA	0.62 ± 0.18	6	
		IL10	Control	1.00 ± 0.28	10	0.74
			MIA	1.13 ± 0.27	9	
		IL1B	Control	1.00 ± 0.14	8	0.61
			MIA	0.88 ± 0.17	9	
		IL6	Control	1.00 ± 0.16	10	0.65
			MIA	1.09 ± 0.13	9	
		STAT3	Control	1.00 ± 0.15	10	0.79
			MIA	1.07 ± 0.23	10	
		TNF	Control	1.00 ± 0.20	10	0.37
			MIA	0.74 ± 0.20	9	
Hypothalamus	21	*IFNG*	Control	0.92 ± 0.15	13	0.88
			MIA	0.89 ± 0.11	17	
		IL10	Control	1.05 ± 0.06	15	0.93
			MIA	1.04 ± 0.09	16	
		IL1B	Control	1.40 ± 0.19	14	0.16
			MIA	1.06 ± 0.15	15	
		IL6	Control	0.97 ± 0.06	15	0.16
			MIA	0.87 ± 0.03	16	
		STAT3	Control	1.07 ± 0.06	15	0.63
			MIA	1.03 ± 0.04	17	
		TNF	Control	1.24 ± 0.09	15	0.85
			MIA	1.21 ± 0.09	17	
		IL1RA	Control	1.04 ± 0.14	15	0.94
			MIA	1.02 ± 0.19	16	

Values are expressed as relative fold change (mean ± SEM). 2-way ANOVA testing for these data, which included sex as a variable, revealed an interaction between MIA and sex ($p = 0.03$) for hippocampal *IL1B* expression, where control females and MIA males had reduced expression compared to control males and MIA females. MIA = maternal immune activation.

Supplemental Table S10. Iba1+ cell soma measurements of each treatment group in the dentate gyrus and hilus region of the hippocampus.

Measurement	7 dpi			21 dpi		
	Control	MIA	p-value	Control	MIA	p-value
Soma Length (µm)	6.3 ± 0.17	6.4 ± 0.28	0.74	7.2 ± 0.16	6.8 ± 0.26	0.27
Soma Width (µm)	3.8 ± 0.07	4.0 ± 0.14	0.20	4.6 ± 0.09	4.4 ± 0.14	0.36
Length-to-Width Ratio	1.7 ± 0.03	1.6 ± 0.02	0.19	1.6 ± 0.03	1.5 ± 0.02	0.50
Estimated Soma Area (μm^2)	19.7 ± 0.72	21.0 ± 1.4	0.39	26.7 ± 1.0	27.6 ± 2.6	0.69

Data are means ± SEM. N = 7-9 fetuses per treatment group at 7 dpi; n = 6-8 fetuses per treatment group at 21 dpi; MIA = maternal immune activation.

Supplemental Table S11. Iba1+ cell soma measurements across gestation in the dentate gyrus and hilus region of the hippocampus.

Measurement	Gestational time point		
	GD 83	**GD 97**	**p-value**
Soma Length (µm)	6.3 ± 0.15	7.0 ± 0.14	**0.002****
Soma Width (µm)	3.9 ± 0.07	4.5 ± 0.08	**<0.0001*****
Estimated Soma Area (µm^2)	20.3 ± 0.73	27.0 ± 1.1	**<0.0001*****
Length-to-Width Ratio	1.63 ± 0.02	1.56 ± 0.02	**0.01***

Data are means ± SEM. Unpaired t tests. N = 14-16 fetuses per gestational time point. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.0001$ (bold); GD = gestational day.

Supplemental Table S12. Iba1+ cell soma measurements of each treatment group in the amygdala.

Measurement	7 dpi			21 dpi		
	Control	MIA	p-value	Control	MIA	p-value
Soma Length (μm)	7.9 ± 0.33	7.7 ± 0.46	0.66	7.9 ± 0.16	7.4 ± 0.05	**0.015***
Soma Width (μm)	5.4 ± 0.24	5.1 ± 0.40	0.56	5.1 ± 0.15	4.9 ± 0.14	0.58
Length-to-Width Ratio	1.5 ± 0.01	1.5 ± 0.04	0.28	1.5 ± 0.02	1.5 ± 0.04	0.20
Estimated Soma Area (μm^2)	36.4 ± 2.7	34.4 ± 4.3	0.70	32.2 ± 1.4	29.1 ± 0.8	0.16

Data are means ± SEM. N = 8 fetuses per treatment group at 7 dpi; n = 5-9 fetuses per treatment group at 21 dpi. * = main effect of maternal treatment, $p < 0.05$ (bold); MIA = maternal immune activation.

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