Supplementary Materials

1. Supplementary methods

### 1.1 Animals and maternal immune activation (MIA)

C57BL/6J mice were used throughout the study and were bred in our facility under a 12-hour light cycle (8am-8pm), with food and water access *ad libitum*. C57BL/6J female and male mice of breeding age (8-12 weeks old) were subject to timed mating procedures. These mice were generated in our facility from parent breeders ordered from the Jackson Laboratories, previously shown not to have Segmented Filamentous Bacteria (SFB), whose presence may be important for enhancing MIA via the maternal IL-17a pathway (1, 2).

One male and one female were placed in a new cage in the afternoon. The following morning the male was removed from the new cage, and the female was checked for the appearance of a seminal plug and weighed. When a seminal plug was observed, this was considered gestational day (GD) 0 (mice were allowed only 1 night together to improve the accuracy of GD 0 detection).

For embryo sample collection, pregnant dams were randomly assigned to one of four treatment groups: (1) poly I:C (P1530-25MG polyinosinic:polycytidylic acid sodium salt TLR ligand tested; Sigma Aldrich) (5mg/kg, intraperitoneally) at gestational day (GD) 9 (POL E; n = 7 dams [4 cohort 1/ 3 cohort 2]), (2) 0.9% sterile NaCl solution at GD 9 (SAL E; n = 7 dams [6 cohort 1/ 1 cohort 2]), (3) poly I:C at GD17 (POL L; n = 7 dams [6 cohort 1/ 2 cohort 2], or (4) saline at GD17 (SAL L; n = 4 dams [2 cohort 1/ 2 cohort 2]).

Collection was performed in two rounds (cohorts) with two different batches of poly I:C (same product). **Table 1** outlines total sample size for embryo groups, with those from cohort 2 in brackets.

In a separate group of dams, poly I:C or saline was injected as described above (GD 9-POL: n=3 batch 1, n=4 batch 2; GD 17-POL n=3 batch 1, n=4 batch 2; GD 9-SAL n=5, GD 17-SAL n=3). Three hours following injection, dams were sacrificed by decapitation without anesthesia, and trunk blood was collected in a 1.5 mL Eppendorf tube. The blood was allowed to coagulate at room temperature for 30 minutes, and then centrifuged for 10 minutes at 4 ℃, with 2000 revolutions per minute. Serum was collected and stored at -80 ℃ until ready for analysis. Serum samples were shipped to the University of Maryland Core Cytokine Facility (http://www.cytokines.com/) for multiplex ELISA to measure levels of IL-6, TNF-α, IL-1β, IL-10 in order to assess the immunostimulatory potential of our poly I:C model. We chose to use a separate group of dams to ensure we could collect enough blood for analysis, and so as not to introduce an additional stressful experience for the dam, thereby potentially confounding the neurodevelopmental trajectory of offspring. Detection ranges were as follows IL-6 (0.64-8000 pg/ml), TNF-α (0.64-3500 pg/ml), IL-1β (0.64-15000 pg/ml), IL-10 (0.64-20000 pg/ml). We have included a timeline of our various sample collections for clarity (**Supplementary Figure 1)**.
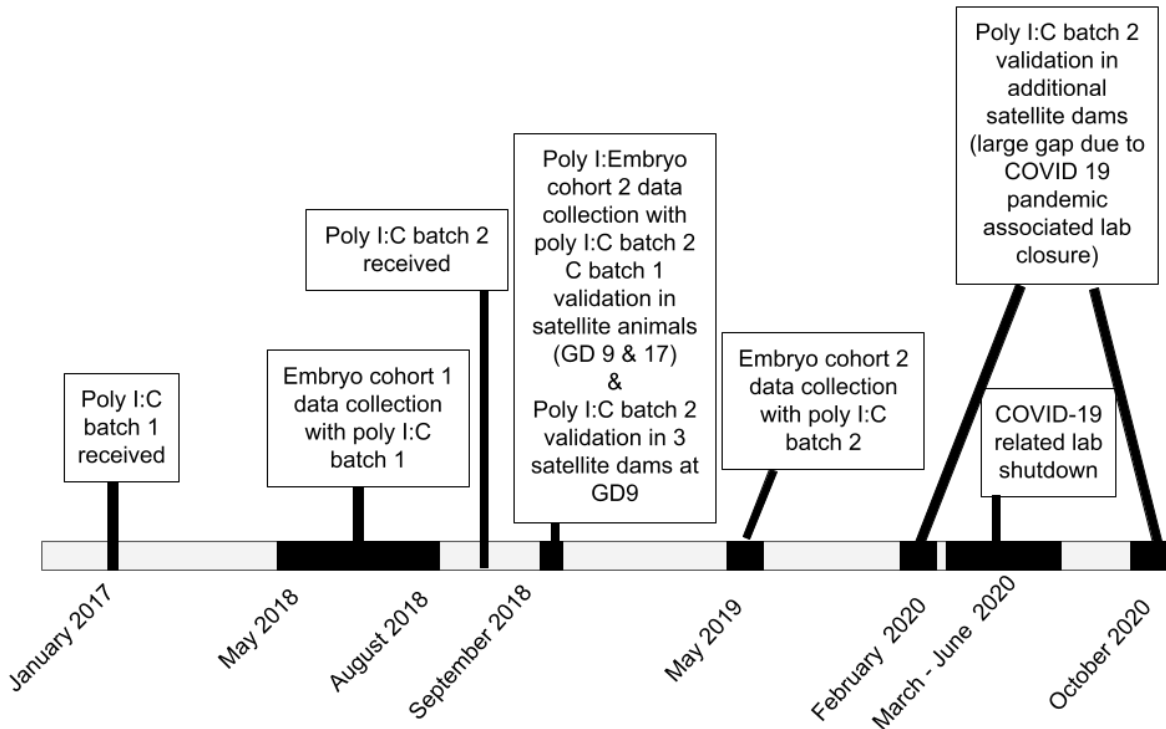
Supplementary Figure 1. Experimental timeline for embryo data collection and poly I:C validation

1.2 Brain sample preparation

Embryos were harvested at GD 18 as follows. Pregnant dams were euthanized by cervical dislocation without anesthesia, embryos were removed and placed in ice cold 1x phosphate buffered saline (PBS) solution. One at a time, embryos were moved into warm (37 °C) PBS, separated from the uterus and placenta, and allowed to bleed out under agitation. A piece of the yolk sac was collected for genotyping for each embryo in order to identify the sex of the mouse via presence of the SRY gene (performed by Transnetyx, Memphis, TN). Once blood was successfully drained, embryos were placed in 4% paraformaldehyde (PFA) with 2% gadolinium (MRI contrast agent; Bracco Imaging S.p.A) in PBS, and post-fixed for 1 week at 4 °C. Finally, embryos were moved to a 2%

gadolinium, 0.02% sodium azide 1x PBS solution for long-term storage at 4 °C until scanning.

1.3 Magnetic resonance image

1.3.1 Acquisition

Prior to imaging, the samples were removed from the contrast agent solution, blotted and placed in 13-mm diameter plastic tubes filled with a proton-free susceptibility-matching fluid (Fluorinert FC-77, 3M Corp. St. Paul, MN). An anatomical scan was performed using a T2-weighted, 3D fast spin echo sequence using a cylindrical k-space acquisition (Nieman, et al., 2005) with TR/TE=350/12 ms, echo train length=6, two averages, field-of-view 20 mm x 20 mm x 25 mm, matrix size=504 x 504 x 630(3). Total imaging time was 14 hours producing images with 40 μm isotropic resolution. MR images of precision-machined phantoms were aligned towards a computed tomography (CT) scan of the same phantom to produce distortion correcting transformations to correct for geometric distortions in a coil-specific manner.

1.3.2 MRI processing

Images were first cropped to center around the head of the embryos, an N4 correction for B1 bias field inhomogeneities(4) and denoising using non-local means (minc_anlm)(5) was applied, and the background was set to zero using minc tools. Next, brain images of all subjects in the study were aligned using the antsMultivariateTemplateConstruction2.sh tool (https://github.com/CoBrALab/twolevel_ants_dbm)(6). Briefly, images were aligned using

rigid registration (translation and rotation), followed by affine (rigid, scaling, and shear), and finally, by nonlinear registration yielding a precise anatomical alignment in an automated, minimally biased fashion, similar to what is done in adult mouse brain studies (7). The output of this iterative registration procedure is a study-specific average against which groups can be compared, as well as deformation fields that map each individual subject to the average at the voxel level. Further, the Jacobian determinants of each deformation field provide a measure of volume difference at each voxel in the image relative to the average (8). Relative Jacobian determinants (used for statistical analysis in this work) explicitly model only the non-linear part of the deformations and remove residual global linear transformations (attributable to differences in total brain size). Prior to performing statistics, Jacobian determinants were blurred at 0.16 mm full-width-at-half-maximum to better conform to Gaussian assumptions for downstream statistical testing.

1.4 Electron microscopy

1.4.1 Dark cell imaging

After post-fixation (described in the main text, **2.3**), tissues were incubated in a mixture (1:1) of 3% ferrocyanide (BioShop, cat# PFC232.250) combined with 4% osmium tetroxide (Electron Microscopy Sciences, cat#19170) for 1 hour, 1% thiocarbohydrazide (in PBS; Electron Microscopy Sciences, cat# 2231-57-4) for 20 minutes, and in 2% osmium tetroxide for 30 minutes. Osmium-thiocarbohydrazide-osmium post-fixed sections were then dehydrated (ethanol (2× in 35%, 1× in 50%, 1× in 70%, 1× in 80%, 1× in 90%, 2× in 100%) followed by 3× in propylene oxide, for 5 minutes each), infiltrated with Durcupan ACM resin (MilliporeSigma, cat# 44611-44614) for 24 hours, and

embedded between two fluoropolymer ACLAR® sheets (Electron Microscopy Sciences, cat# 50425-25) at 55-60 °C for 5 days. Using a binocular microscope, the dorsal hippocampus was excised from tissue sections using a razor blade, glued with Ultra Gel Control super glue (base of ethylene cyanoacrylate, LePage) onto a resin block, and left to incubate overnight at 55-60 °C. Next, the resin block was trimmed with a razor blade into a pyramidal shape to allow for more stable and precise sectioning. Following glue removal, tissue was sectioned into ~70-75 nm ultrathin sections using an Ultracut UC7 ultramicrotome (Leica Biosystems). Three levels of section-rubans were collected at an interval of 10 μm, which represented different levels of the dorsal hippocampus. Section-rubans were collected on a silicon nitride chip and glued on a specimen mount and imaged by array tomography at 25 nm resolution with an acceleration voltage of 1.4 kV and current of 1.2 nA using a Crossbeam 540 Gemini scanning electron microscope (Zeiss).

Images from all treatment groups were analyzed blind to the experimental conditions using QuPath software (v0.2.0-m3) (9). For each picture, region areas were traced using the "polygon" tool and measured to determine cell density. Total cell numbers (identified by a well-defined nucleus), dark cells (neuronal and glial cells), and apoptotic cells were then counted within the dorsal hippocampus (CA1, CA3, and dentate gyrus). Data were reported both as a measure of cell density (divided by the region area) or of cell population percentage (divided by the total cell count).

Dark cells were identified by their darker cytoplasm and dark nucleus with a faint heterochromatine pattern (10–12). Dark neurons were distinguished by their often enlarged cell cytoplasm with a visible apical dendrite (12) (see

http://www.bu.edu/agingbrain/chapter-1-neuronal-cell-bodies-2 for more detail) . Of note, some dark neurons were, however, shrunken, extended and deformed but recognized by their pyknotic nuclei (11). Glial cell bodies were often smaller in size than dark neurons as well as less regular with, occasionally, several smaller irregular protrusions. Moreover, in contrast to dark neurons, dark glial cell nuclei possess a distinct faint heterochromatin pattern, showing a thin rim of more condensed chromatin at the edge of the neuropil or clumped patches of chromatin (13). Lastly, apoptotic cells or dying cells were identified by a pyknotic or fragmented nucleus, a distended or incomplete nuclear envelope and an accumulation of autophagic vacuoles in their cytoplasm (13, 14)(13)(13, 14).

1.4.2. Active Caspase-3 staining and imaging

Sections were washed in PBS. Sections were placed in a sealed well-plate, quenched in citrate acid buffer for 40 minutes, floating in a water bath at 70°C. After reaching room temperature, sections were washed with PBS and quenched with 0.3% H_2O_2 (in PBS) for 5 minutes and washed again in PBS. The sections were then incubated in $NABH_4$ 0.1% (in PBS) for 30 minutes, washed with PBS and incubated 1 hour in blocking buffer (BB) (5% natural goat serum, 0.5% fish gelatin and 0.01% Triton X-100). Samples were then incubated overnight in primary antibody, rabbit anti-active caspase-3 [1:400] (BD Pharmigen, cat# 559565) in BB at 4°C. The next day samples were wash in Tris-buffered saline (TBS), incubated for 1.5 hours in secondary antibody, biotinylated goat-anti-rabbit (Jackson ImmunoResearch cat# 111-066-046, West Grove, PA, United States). Samples were washed in TBS, followed by an incubation in avidin-biotin complex (1:1:100 in TBS; cat# PK-6100, Vector Laboratories, Burlingame, CA, United States) for

1 hour at RT, then washed and revealed in 0.05% diaminobenzidine (DAB, 0.015% H2O2, in TBS; cat# D5905-50TAB, MilliporeSigma) and washed in PB. OTO post-fixation was done as previously discussed in the methods. After OTO post-fixation, ultrathin sections were cut at 70nm using a Leica ARTOS 3D Ultramicrotome and pictures were acquired using a JOEL JEM-1400 transmission electron microscope operated at 80 kV and equipped with a Gatan SC-1000 digital camera.

1.5 Statistical analyses

1.5.1 Neuroimaging analysis

Our first goal was to determine whether there were neuroanatomical differences in our two control groups, SAL E (GD9) and SAL L (GD17). We ran a whole-brain (delimited by a brain mask) voxel-wise linear mixed effects model on the relative Jacobian determinant files of each subject with injection timing and sex as fixed effects, and litter and number of pups per litter as random intercept to account for litter-specific variation. A False Discovery Rate (FDR) correction was applied. There were no differences, and SAL E and SAL L offspring were combined and set as the reference group. Finally, sex differences were explored, investigating the interaction between group and sex, with the same covariates as above. The statistical models applied to the relative Jacobian determinants to assess for group differences, described in **X** are detailed below:

Main model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL\_E_{subject,j} + \beta_3 groupPOL\_L_{subject,j} + \mathbf{b_1} cohort + \mathbf{b_2} litter\ size + \epsilon_{subject,j}$

Sex interaction model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL\_E_{subject,j} +$

$\beta_3 groupPOL\_L_{subject,j} + \beta_4 sexF:groupPOL\_E_{subject,j} + \beta_5 sexF:groupPOL\_L_{subject,j}$

$+ \mathbf{b_1} cohort + \mathbf{b_2} litter\ size + \epsilon_{subject,j}$

Y= outcome measures (i.e., blurred absolute Jacobian determinants); β_i= fixed effect coefficient; β_0 = equation intercept; **b** = random predictor; $\boldsymbol{\epsilon}$ = random error; **j** = repeated measure per subject; : = interaction; POL_E = early polyI:C group relative to SAL as reference; POL_L = late polyI:C group relative to SAL as reference; SexF = female sex relative to male as reference

As an exploratory analysis, we investigated whether there were any volume differences in the organs due to GD9 or 17 MIA exposure. We ran the same model as above on the voxels within the organ cavity defined manually with the same models described above.

2. Supplementary results

2.1 Effect of poly I:C injection on maternal cytokines

We observed an increase in levels of pro-inflammatory cytokines IL-6, IL-1β, and IL-10, but not TNF-α, in a separate cohort of pregnant dams 3-hours post poly I:C injection with our first batch on GD 9 relative to saline control on GD 9. For the second batch of poly I:C injected on GD 9, all 4 pro-inflammatory cytokines were increased (IL-6, IL-1β, TNF-α) 3 hours post-injection, but the anti-inflammatory cytokine IL-10 was not.

Exposure to our first batch of poly I:C (batch 1) on GD 17 increased levels of pro-inflammatory cytokines IL-6 relative to all but one SAL L dam which had exceptionally high IL-6 values. No differences were observed for TNF-α, IL-1β, or IL-10 relative to GD 17 saline controls. Our second batch of poly I:C similarly only increased IL-6, and had no effect on the other cytokine levels, however, for IL-1β and IL-10 values were below the detection threshold (<0.64) (**Supplementary Table 1** and Supplementary **Figure 2).** Additionally, our second batch of poly I:C was tested at two separate timepoints in satellite dams, showing a degradation of the product, and decreased immunostimulatory potential over time (**Supplementary Figure 3).**
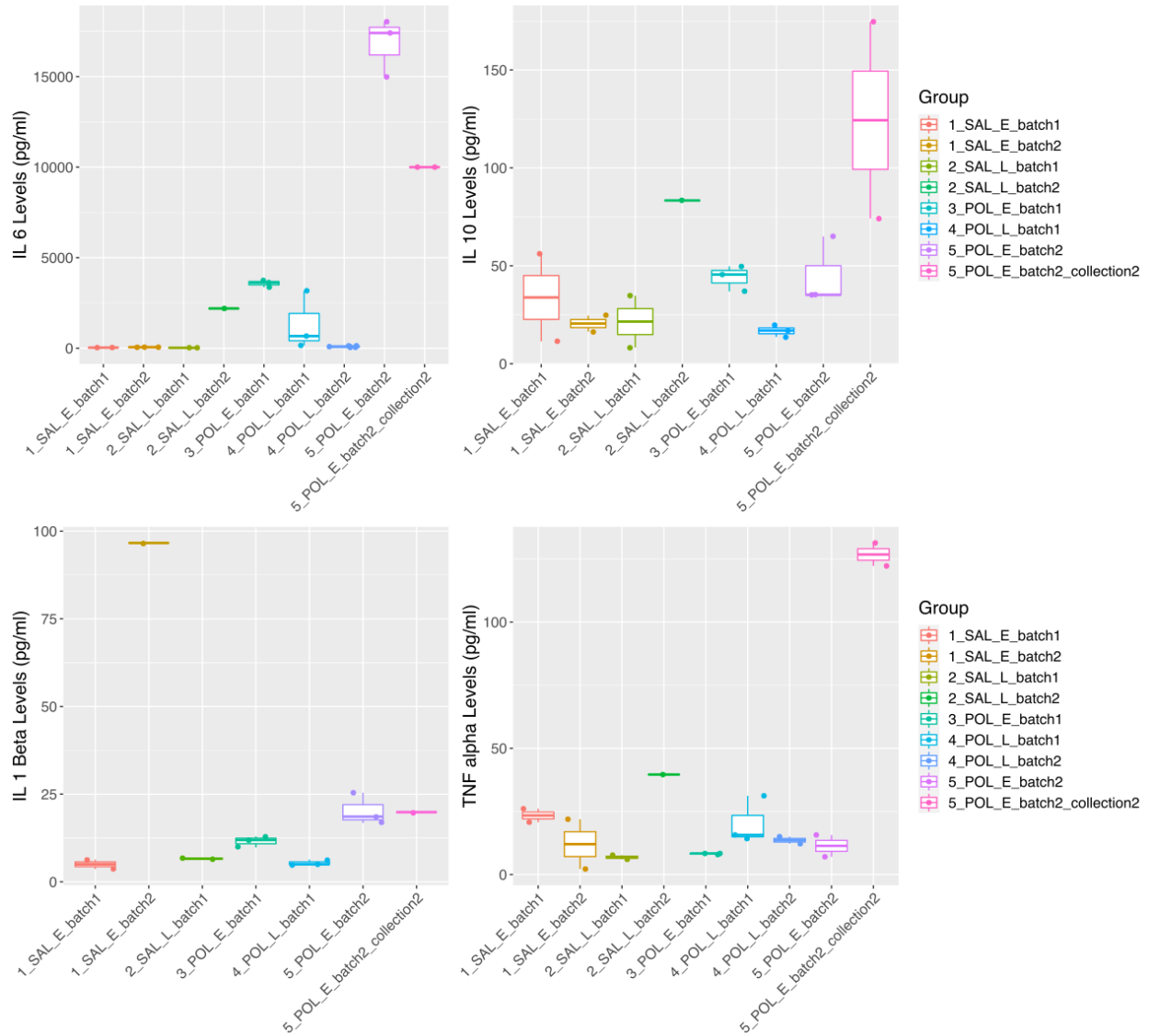
Supplementary Figure 2. Cytokine profile for validation of poly I:C with values under detection threshold removed. Batch 1 of poly I:C was used to collect data for our first cohort, whereas batch 2 was used for our second cohort. Batch 2 was tested at two separate times (due to delays associated with the COVID-19 pandemic).

Cytokine Response
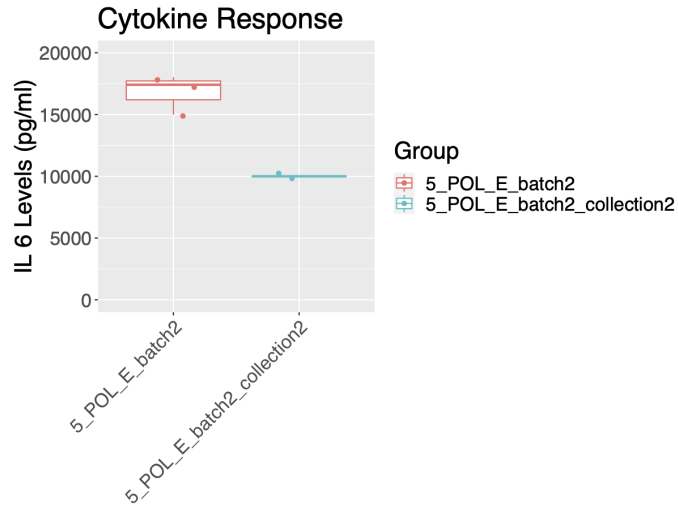
Supplementary Figure 3. Comparing poly I:C immunostimulatory potential either 1 few months after arrival (red), or 1.5 years after arrival (blue). This shows that the potency degrades over time, but that it still elicits an immune response.

Supplementary Table 1. Cytokine profile for validation of poly I:C with values under detection threshold removed presented as mean and [range]. Batch 1 of poly I:C was used to collect data for our first cohort, whereas batch 2 was used for our second cohort. Batch 2 was tested at two separate times (due to delays associated with the COVID-19 pandemic).

	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-α (pg/ml)	IL-1β (pg/ml)
SAL E (n=5)	45.54 [30.58-56.59]	27.17 [16.64-56.22]	17.71 [2.18-26.05]	35.54 [3.65 - 96.2]
SAL L (n=3)	746.33 [22.2-2192.47]	42.13 [8.30-83.37]	17.76 [5.99-39.59]	6.61 [6.34-6.88]
POL E batch 1 (n=3)	3578.84 [3624.87-3740.81]	44.05 [36.93-49.70]	8.20 [7.91-8.35]	11.56 [9.80-12.95]
POL E batch 2, collection 1 (2018) (n=3)	16810.37 [14985.47-18033.31]	45.11 [35.18-64.96]	11.35 [7.02-15.67]	20.26 [16.78-25.40]
POL_E batch 2, collection 2 (2020) (n=2)	10000 [1000-1000]	124.32 [74.20-174.44]	126.76 [122.21-131.31]	19.86
POL L batch 1 (n=3)	1298.69 [54.36-3173.25]	16.73 [13.67-19.58]	20.35 [14.21-31.18]	5.45 [5.00-6.34]
POL L new (n=6 for IL-6, n=2 for TNF-A)	87.41 [45.59-131.44]	NA	13.58 [12.18-14.98]	NA

2.2 MRI results

2.2.1 Difference between POL E and POL L

Significant brain volume differences were also observed between the POL E and POL L offspring ((t=3.590, <1%FDR) wherein the POL L offspring also had larger brain volume than POL E Offspring (not just SAL) in may similar regions such as, but not limited to, the left caudate, the bilateral dorsal hippocampus, the bilateral medial preoptic area, the bilateral septal nucleus (posterior), the anterior commissure, external capsule, and centromedian thalamus (as well as other thalamic nuclei). The POL L offspring had smaller volume than POL E offspring in the anterior septal nucleus, the cingulum/hippocampus, with mixed results in the cerebellum (some increased regions and some decreased) (**Supplementary figure 4**).
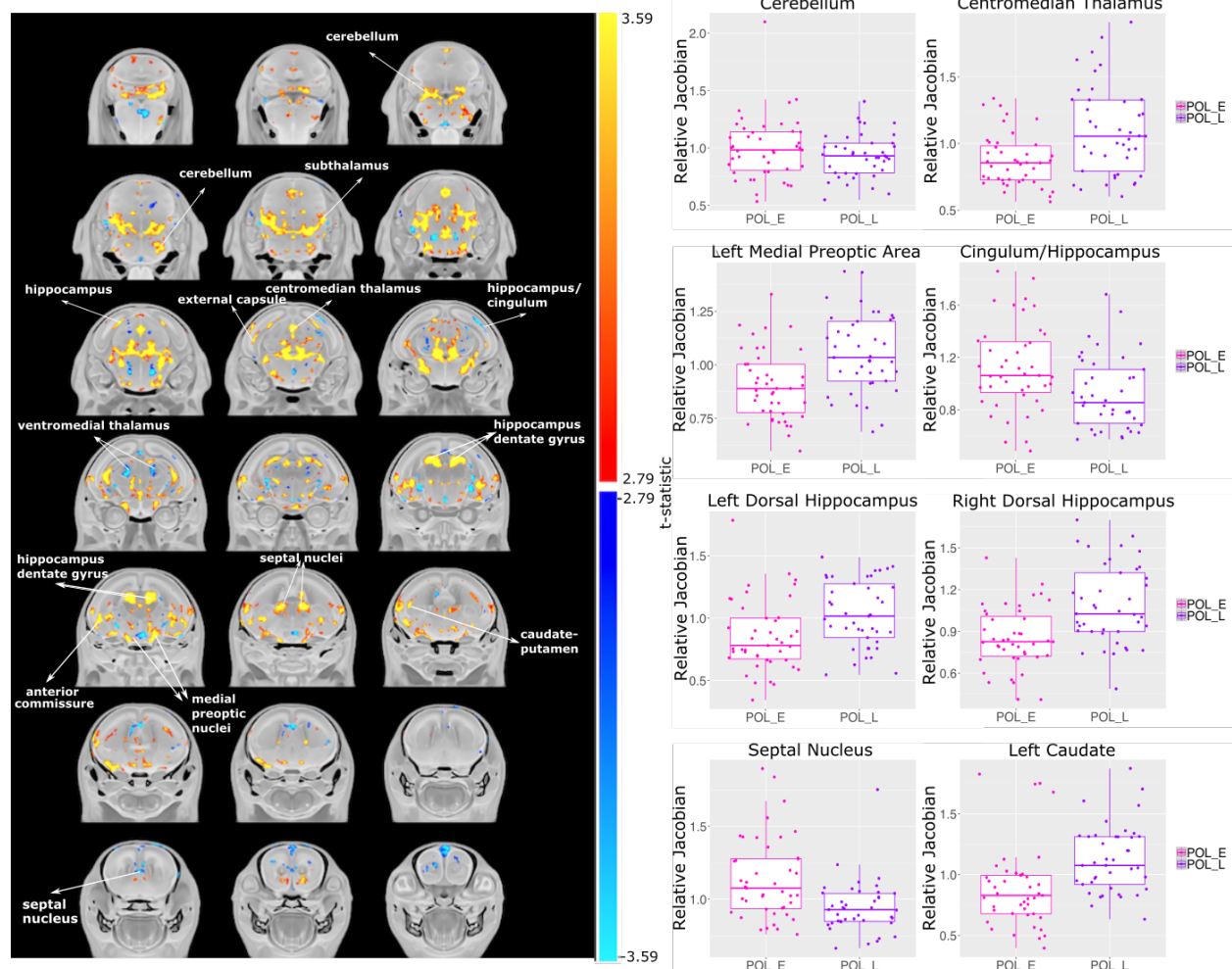
Supplementary Figure 4. Neuroanatomical differences due to early (GD9) or late (GD17) MIA-exposure in the GD 18 embryo mouse brain. **A.** t-statistic map of group (POL L vs POL E) thresholded at 5% (bottom, t=2.79) and 1% FDR (top, t=3.59) overlaid on the study average. **B.** Boxplots of peak voxels (voxels within a region of volume change showing largest effect) selected from regions of interest highlighted in white text in **A**. For all boxplots, the relative Jacobian determinants are plotted on the y-axis as in Figure 2 and 3.

2.2.2. No volumetric differences in the embryo organs

No significant voxel-wise differences were observed in the volume of the organ cavity for POL E or POL L exposed embryos at GD 18. The average for the whole-body registration is presented below in **Supplementary figure 5.**
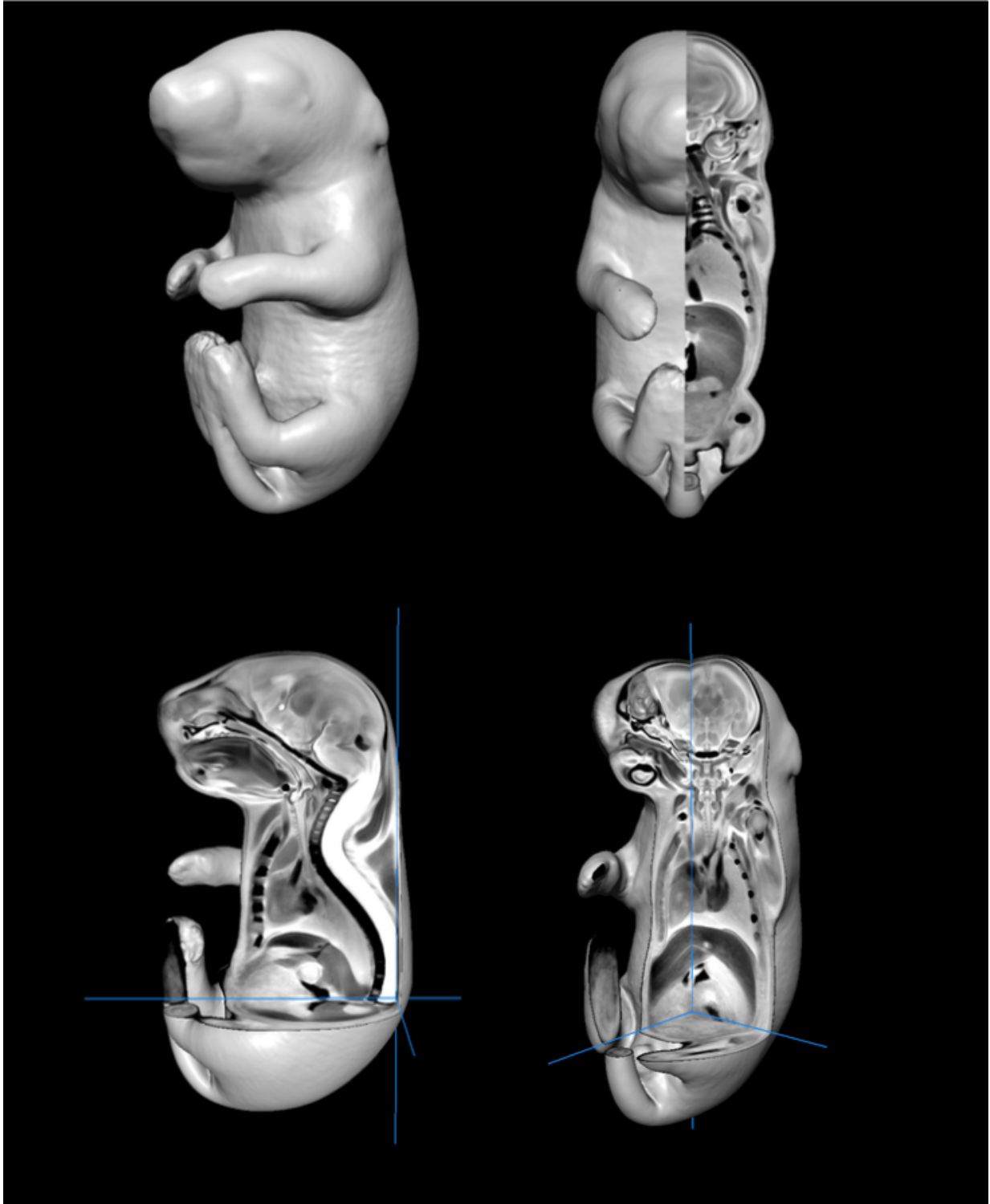
Supplementary figure 5. Representative 3D view of whole-body nonlinear average for the GD 18 embryo (40 µm^3 resolution).

2.3 Electron microscopy results

2.3.1 No differences in total cell density

As discussed in the main paper section 3.2, there were no differences in total cell density (chi-squared = 0.68038, df = 2, p-value = 0.7116; **Supplementary figure 6**). Further representative sections displayed in **Supplementary figure 7** per cell type per group.
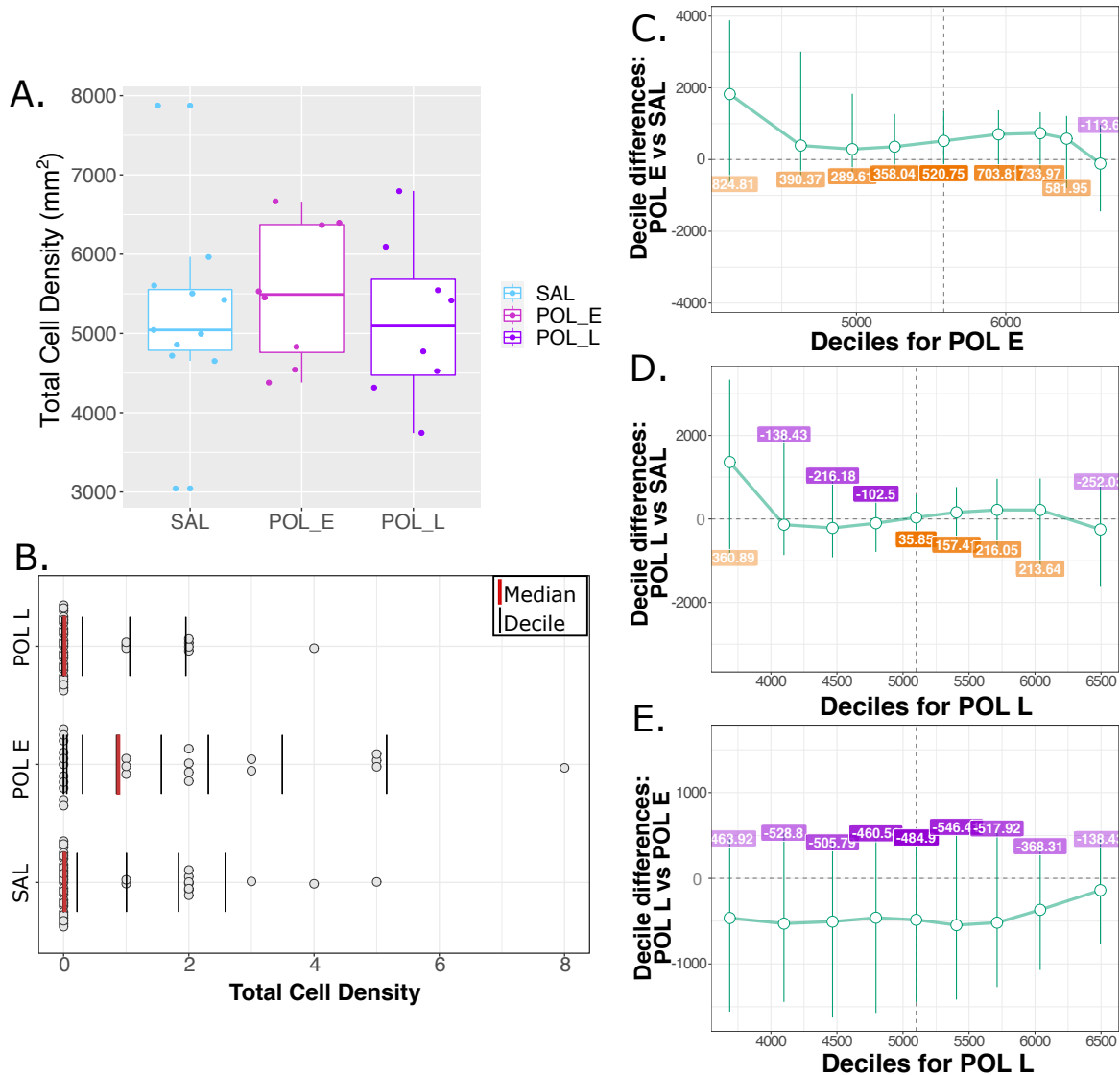
Supplementary figure 6. No differences in total cell density between groups. **A.** Boxplot showing group differences between SAL, POL E, and POL L offspring total cell density in the dorsal hippocampus. **B.** Distribution plot in which the red line identifies the median of the data,

while each black bar denotes a decile of distribution. **C, D, and E** show results from the percentile bootstrapping technique applied to identify the difference in decile between the POL E and SAL **(C)**, POL L and SAL **(D)**, and POL E and POL L **(E)**.
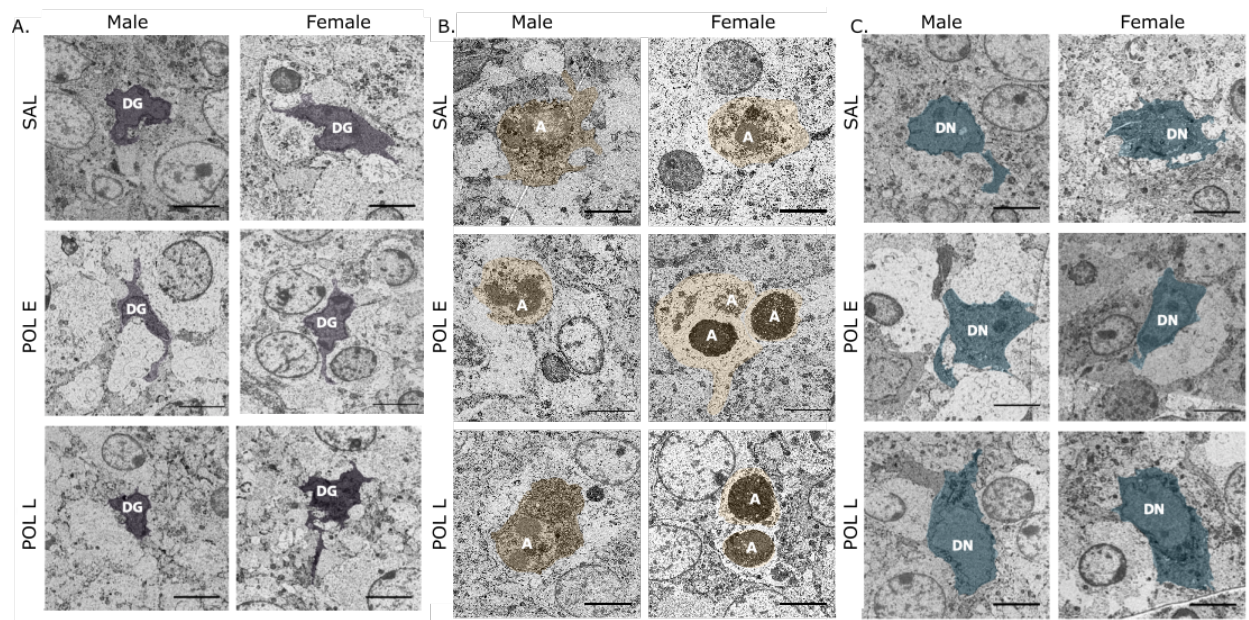
Supplementary figure 7. Image acquired by scanning electron microscopy (25 nm resolution) in the dorsal hippocampus from representative offspring (equivalent to coronal slice 14 from **Figure 4A**) highlighting dark glial cells (DG) in the left panel, apoptotic cells (A) in the middle panel, and dark neurons (DN) in the rightmost panel. To aid with visibility, apoptotic cells are pseudo-coloured in pale orange and dark neurons in dark blue. Scale bar equivalent to 5 µm.

2.3.2 Sex differences in distribution

As was done for the MRI data, sex differences were investigated as a secondary, exploratory analysis. For total cell density there were largely no differences in distribution for POL E males (apart from lower density at the first decile for POL E males, p=0.05) nor POL L males relative to SAL (apart from lower density at the first decile for POL L males, p=0.019), nor for POL L males relative to POL E males. POL E females did have slightly higher density than both SAL (deciles 1-6, p<0.038) and POL L females (deciles 1-4,

p<0.05) while POL L females were no different than SAL females (**Supplementary Tables 14-19; Supplementary Figure 8AD).**

A greater sex-dependence emerged with the dark glial cell density, wherein POL L females had significantly higher density than SAL and POL E females across all deciles of the distribution (p<0.015). Further, POL E females had even lower levels of glial cell density than SAL across higher deciles of the distribution (deciles 5-9, p<0.041). In contrast, POL L males had lower density than SAL males at lower deciles of distribution (deciles 1-6, p<0.048), and lower density than POL E males at the first decile (p=0.29). Similar to the females, POL E males also had lower density than SAL at higher deciles (deciles 5-9, p<0.029) (**Supplementary Tables 20-26; Supplementary Figure 8EH)**.

For dark neurons, both POL E males had lower density than SAL at the higher deciles of distribution (deciles 5-9, p<0.037), while POL L males had lower density than SAL at the lower deciles (deciles 1-6, p<0.008). There were no differences in distribution between POL L and POL E males, nor for any of the female pairings (**Supplementary Tables 27-32; Supplementary Figure 8IL)**.

Finally, for apoptotic cells, POL E females had significantly higher density across most deciles of the distribution relative to SAL (deciles 107, p<0.044), and across all deciles relative to POL L (p<0.005). Males instead showed no differences for any pairwise comparisons (**Supplementary Tables 33-37; Supplementary Figure 8MP).**
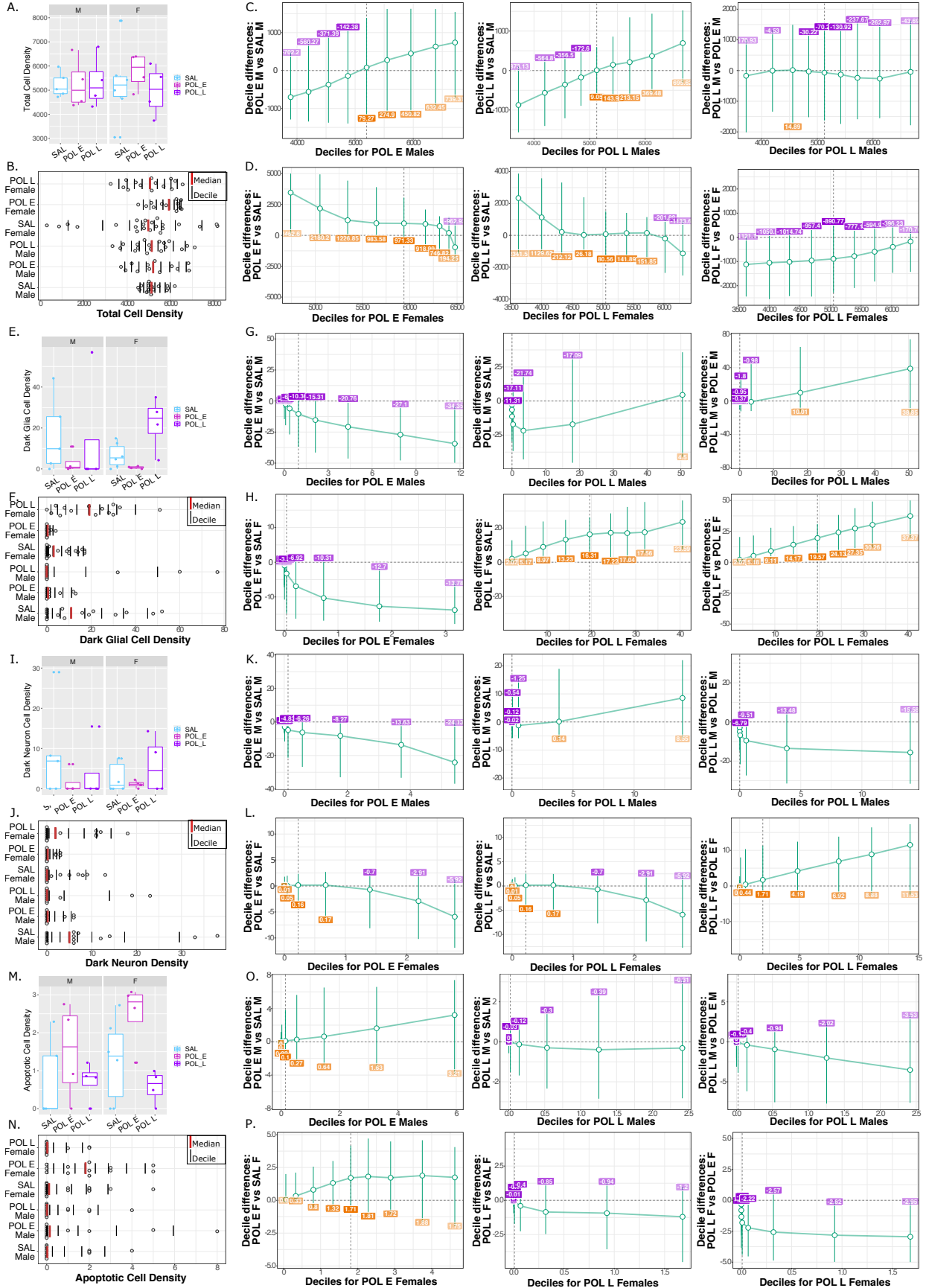
Supplementary figure 8. Differences in distribution of dark glial, dark neuron, and apoptotic cell density per sex and per group. **A.** Boxplot showing total cell density (per mouse) per sex per group (n=3-4/sex/group). **B**. Distribution of total cell density for all hippocampal slices per animal for each sex/group. The red line identifies the median of the data, while each black bar denotes a decile of distribution. A percentile bootstrapping technique applied to identify the difference in decile between the POL E and SAL, POL L and SAL, and POL E and POL L for males (**C**) and females (**D)** showing generally no differences. **E.** Boxplot showing dark glial cell density (per mouse) per sex per group (n=3-4/sex/group). **F**. Distribution of dark glial cell density for all hippocampal slices per animal for each sex/group. Difference in decile between all groups for males (**G**) and females (**H**) wherein POL L females had higher density than SAL and POL E, and POL E had lower density than SAL and POL L. **I.** Boxplot showing dark neuron density (per mouse) per sex per group (n=3-4/sex/group). **J.** Distribution of dark neuron density for all hippocampal slices per animal for each sex/group. Difference in decile between all groups for males (**K**) and females (**L**). **M.** Boxplot showing apoptotic cell density (per mouse) per sex per group (n=3-4/sex/group). **N.** Distribution of apoptotic cell density for all hippocampal slices per animal for each sex/group. Difference in decile between all groups for males (**O**) and females (**P**).

Supplementary tables

For supplementary tables **2-37**, CI (confidence interval), p crit (uncorrected p-value), p-value (bootstrap corrected p-value); SAL (saline); POL E (early poly I:C group); POL L (late poly I:C group).

Supplementary Table 2. Total Cell Density POL E vs SAL difference

Decile	POL E	SAL	Difference	CI lower	CI upper	p crit	p-value
1	4151.408	2326.602	1824.8056	-685.2851	3881.0665	0.00277778	0.092
2	4627.183	4236.812	390.3706	-557.2165	3007.9732	0.00833333	0.358
3	4972.235	4682.629	289.6055	-464.8035	1833.0812	0.00555556	0.286
4	5255.654	4897.611	358.0427	-386.0938	1266.3072	0.00416667	0.190
5	5584.768	5064.015	520.7527	-381.3019	1342.328	0.00238095	0.083
6	5950.95	5247.137	703.8125	-377.1873	1373.1058	0.00208333	0.066
7	6230.605	5496.634	733.9711	-382.9449	1320.4004	0.00185185	0.045
8	6407.253	5825.298	581.955	-793.8431	1215.9668	0.00333333	0.136
9	6633.453	6747.055	-113.602	-1442.54	964.3226	0.01666667	0.892

Supplementary Table 3. Total Cell Density POL L vs SAL difference

Decile	POL L	SAL	Difference	CI lower	CI upper	p crit	p-value
1	3687.489	2326.602	1360.88663	-933.4781	3332.1491	0.00555556	0.278
2	4098.382	4236.812	-138.4302	-861.9588	2023.1686	0.025	0.848

3	4466.444	4682.629	-216.18463	-917.2317	1036.4123	0.00714286	0.537
4	4795.114	4897.611	-102.49719	-788.0476	604.2014	0.0125	0.764
5	5099.865	5064.015	35.85004	-488.4449	579.8772	0.05	0.894
6	5404.544	5247.137	157.40696	-617.1548	763.2366	0.01	0.648
7	5712.688	5496.634	216.05434	-729.4657	961.1043	0.00625	0.495
8	6038.94	5825.298	213.64173	-1196.4319	968.0088	0.00833333	0.623
9	6495.022	6747.055	-252.03283	-1624.8705	897.5734	0.01666667	0.732

Supplementary Table 4. Total Cell Density POL L vs POL E difference

Decile	POL L	POL E	Difference	CI lower	CI upper	p crit	p-value
1	3687.489	4151.408	-463.9189	-1557.1103	465.3654	0.00185185	0.112
2	4098.382	4627.183	-528.8008	-1442.7045	531.7885	0.00416667	0.155
3	4466.444	4972.235	-505.7901	-1625.6035	419.6274	0.00277778	0.151
4	4795.114	5255.654	-460.5399	-1571.5615	525.1342	0.00333333	0.160
5	5099.865	5584.768	-484.9026	-1446.6147	476.4443	0.00555556	0.195
6	5404.544	5950.95	-546.4056	-1416.1051	600.4311	0.00238095	0.189
7	5712.688	6230.605	-517.9168	-1268.8219	582.2052	0.00208333	0.136
8	6038.94	6407.253	-368.3133	-1071.0121	375.3764	0.00833333	0.184
9	6495.022	6633.453	-138.4309	-772.0971	459.7784	0.01666667	0.625

Supplementary Table 5. Total Dark Glial Cell Density POL E vs SAL difference

Decile	POL E	SAL	Difference	CI lower	CI upper	p crit	p-value
1	1.56E-08	1.65E-05	-1.65E-05	-0.117288	0.0008025	0.00625	0.283
2	7.42E-06	6.43E-03	-6.43E-03	-2.801359	0.07893029	0.003125	0.201
3	6.21E-04	2.32E-01	-2.32E-01	-6.841321	0.29481655	0.00208333	0.109
4	1.56E-02	1.88E+00	-1.87E+00	-10.613885	0.4750728	0.0015625	0.067
5	1.48E-01	5.73E+00	-5.58E+00	-14.32128	0.93706151	0.00125	0.020
6	6.36E-01	1.01E+01	-9.51E+00	-17.339485	-0.2218018	0.00104167	0.001
7	1.59E+00	1.44E+01	-1.28E+01	-23.66778	-2.6836544	0.00078125	<0.001
8	3.54E+00	1.85E+01	-1.50E+01	-39.659531	-2.5981535	0.00089286	<0.001
9	8.55E+00	3.11E+01	-2.25E+01	-45.872087	-4.508818	0.00069444	<0.001

Supplementary Table 6. Total Dark Glial Cell Density POL L vs SAL difference

Decile	POL L	SAL	Difference	CI lower	CI upper	p crit	p-value
1	7.35E-07	1.56E-08	7.20E-07	-0.0091239	0.07514775	6.94E-04	0.562
2	4.50E-04	7.42E-06	4.42E-04	-0.3304835	1.09187281	3.47E-04	0.478
3	3.15E-02	6.21E-04	3.08E-02	-0.3715421	8.08383966	2.31E-04	0.339
4	5.27E-01	1.56E-02	5.11E-01	-1.2459148	13.9375972	1.74E-04	0.241
5	3.24E+00	1.48E-01	3.09E+00	-2.386622	24.4702609	1.39E-04	0.124
6	1.03E+01	6.36E-01	9.67E+00	-2.377137	28.7006809	1.16E-04	0.043

7	2.06E+01	1.59E+00	1.90E+01	-1.7454353	39.9903739	9.92E-05	0.008
8	2.97E+01	3.54E+00	2.62E+01	1.16311037	56.2422815	8.68E-05	<0.001
9	4.54E+01	8.55E+00	3.68E+01	7.18141072	70.1908463	7.72E-05	<0.001

Supplementary Table 7. Total Dark Glial Cell Density POL L vs POL E difference

Decile	POL L	POL E	Difference	CI lower	CI upper	p crit	p-value
1	7.35E-07	1.65E-05	-1.58E-05	-0.0408415	0.01028092	0.01666667	0.634
2	4.50E-04	6.43E-03	-5.98E-03	-1.2774581	0.50064786	0.01	0.597
3	3.15E-02	2.32E-01	-2.01E-01	-5.6629882	4.09638211	0.00833333	0.531
4	5.27E-01	1.88E+00	-1.36E+00	-9.0806618	8.04585178	0.0125	0.542
5	3.24E+00	5.73E+00	-2.49E+00	-11.367416	12.7351975	0.025	0.683
6	1.03E+01	1.01E+01	1.55E-01	-11.178672	15.8678074	0.05	0.968
7	2.06E+01	1.44E+01	6.23E+00	-13.049187	24.0356019	0.00714286	0.435
8	2.97E+01	1.85E+01	1.12E+01	-14.425163	34.1453	0.00555556	0.181
9	4.54E+01	3.11E+01	1.43E+01	-15.408127	48.9480906	0.00625	0.255

Supplementary Table 8. Total Dark Neuron Density POL E vs SAL difference

Decile	POL E	SAL	Difference	CI lower	CI upper	p crit	p-value
1	9.72E-11	4.80E-10	-3.83E-10	-4.15E-05	1.11E-05	0.025	0.851
2	1.15E-07	2.01E-06	-1.90E-06	-1.95E-02	5.61E-03	0.0125	0.657

3	2.37E-05	6.84E-04	-6.60E-04	-7.39E-01	1.49E-01	0.00833333	0.554
4	1.43E-03	4.08E-02	-3.94E-02	-3.36E+00	5.12E-01	0.00625	0.438
5	3.13E-02	6.07E-01	-5.76E-01	-5.59E+00	1.26E+00	0.005	0.299
6	2.81E-01	2.83E+00	-2.55E+00	-6.92E+00	1.87E+00	0.00416667	0.191
7	1.15E+00	5.58E+00	-4.44E+00	-9.81E+00	1.42E+00	0.00357143	0.051
8	2.59E+00	8.05E+00	-5.47E+00	-2.00E+01	4.47E-01	0.003125	0.010
9	4.56E+00	1.55E+01	-1.09E+01	-3.14E+01	-1.82E+00	0.00277778	<0.001

Supplementary Table 9. Total Dark Neuron Density POL L vs SAL difference

Decile	POL L	SAL	Difference	CI lower	CI upper	p crit	p-value
1	6.46E-14	4.80E-10	-4.80E-10	-1.61E-04	1.36E-07	0.0125	0.3585
2	7.49E-10	2.01E-06	-2.01E-06	-2.45E-02	1.10E-04	0.01	0.330
3	9.04E-07	6.84E-04	-6.83E-04	-6.13E-01	2.90E-02	0.00714286	0.339
4	2.21E-04	4.08E-02	-4.06E-02	-3.24E+00	4.67E-01	0.00625	0.275
5	1.46E-02	6.07E-01	-5.93E-01	-5.25E+00	2.81E+00	0.00555556	0.287
6	3.15E-01	2.83E+00	-2.52E+00	-6.90E+00	5.72E+00	0.00833333	0.304
7	2.51E+00	5.58E+00	-3.08E+00	-8.49E+00	6.47E+00	0.01666667	0.479
8	8.10E+00	8.05E+00	4.66E-02	-9.50E+00	7.19E+00	0.05	0.936
9	1.50E+01	1.55E+01	-4.46E-01	-1.90E+01	9.97E+00	0.025	0.860

Supplementary Table 10. Total Dark Neuron Density POL L vs POL E difference

Decile	POL L	POL E	Difference	CI lower	CI upper	p crit	p-value
1	6.46E-14	9.72E-11	-9.71E-11	-0.0011336	4.43E-06	0.00039683	0.533
2	7.49E-10	1.15E-07	-1.14E-07	-0.017834	7.33E-04	0.00055556	0.588
3	9.04E-07	2.37E-05	-2.28E-05	-0.2128114	6.68E-02	0.00069444	0.706
4	2.21E-04	1.43E-03	-1.21E-03	-1.1878761	9.77E-01	0.00092593	0.785
5	1.46E-02	3.13E-02	-1.67E-02	-2.1454795	3.32E+00	0.00277778	0.910
6	3.15E-01	2.81E-01	3.39E-02	-2.7517723	8.25E+00	0.00138889	0.905
7	2.51E+00	1.15E+00	1.36E+00	-3.808122	1.18E+01	0.00046296	0.576
8	8.10E+00	2.59E+00	5.51E+00	-5.1950725	1.67E+01	0.00034722	0.230
9	1.50E+01	4.56E+00	1.05E+01	-4.0620577	1.91E+01	0.00030864	0.016

Supplementary Table 11. Total Apoptotic Cell Density POL E vs SAL difference

Decile	POL E	SAL	Difference	CI lower	CI upper	p crit	p-value
1	2.98E-05	3.20E-14	2.98E-05	-4.43E-07	0.07461499	0.00069444	0.013
2	2.84E-03	6.63E-10	2.84E-03	-1.04E-03	0.85163803	0.00078125	0.020
3	5.05E-02	1.04E-06	5.05E-02	-3.11E-02	1.35800997	0.00089286	0.028
4	3.03E-01	2.62E-04	3.03E-01	-1.75E-01	2.33253458	0.00104167	0.039
5	8.67E-01	1.45E-02	8.53E-01	-7.87E-01	2.84572975	0.0015625	0.064

6	1.56E+00	2.16E-01	1.35E+00	-1.01E+00	3.73112757	0.003125	0.124
7	2.31E+00	1.00E+00	1.31E+00	-1.04E+00	4.21302706	0.00625	0.121
8	3.49E+00	1.84E+00	1.66E+00	-9.74E-01	4.89217444	0.00208333	0.083
9	5.16E+00	2.58E+00	2.58E+00	-1.13E+00	5.81488105	0.00125	0.057

Supplementary Table 12. Total Apoptotic Cell Density POL L vs SAL difference

Decile	POL L	SAL	Difference	CI lower	CI upper	p crit	p-value
1	6.66E-16	3.20E-14	-3.13E-14	-1.62E-08	7.86E-10	0.05	0.727
2	1.39E-11	6.63E-10	-6.49E-10	-3.36E-05	7.97E-06	0.025	0.654
3	2.78E-08	1.04E-06	-1.01E-06	-9.36E-03	1.07E-03	0.01666667	0.654
4	1.10E-05	2.62E-04	-2.51E-04	-2.28E-01	3.02E-02	0.0125	0.626
5	1.11E-03	1.45E-02	-1.34E-02	-8.71E-01	2.40E-01	0.01	0.534
6	3.25E-02	2.16E-01	-1.83E-01	-1.64E+00	7.34E-01	0.00833333	0.470
7	2.99E-01	1.00E+00	-7.05E-01	-1.93E+00	1.17E+00	0.00714286	0.398
8	1.06E+00	1.84E+00	-7.78E-01	-2.32E+00	1.25E+00	0.00555556	0.288
9	1.95E+00	2.58E+00	-6.31E-01	-3.03E+00	1.40E+00	0.00625	0.353

Supplementary Table 13. Total Apoptotic Cell Density POL L vs POL E difference

Decile	POL L	POL E	Difference	CI lower	CI upper	p crit	p-value
1	6.66E-16	2.98E-05	-2.98E-05	-0.3070457	3.17E-08	0.00013889	0.006

2	1.39E-11	2.84E-03	-2.84E-03	-0.9555023	6.64E-06	0.00015625	0.003
3	2.78E-08	5.05E-02	-5.05E-02	-1.8488396	9.25E-04	0.0003125	0.008
4	1.10E-05	3.03E-01	-3.03E-01	-2.4652326	2.24E-02	0.00025	0.009
5	1.11E-03	8.67E-01	-8.66E-01	-2.8238152	1.43E-01	0.000625	0.015
6	3.25E-02	1.56E+00	-1.53E+00	-3.9222594	4.22E-01	0.00125	0.020
7	2.99E-01	2.31E+00	-2.01E+00	-4.9137293	7.29E-01	0.00020833	0.011
8	1.06E+00	3.49E+00	-2.43E+00	-6.3036623	2.18E-01	0.00041667	0.007
9	1.95E+00	5.16E+00	-3.21E+00	-6.6126179	1.50E+00	0.00017857	0.014

Supplementary Table 14. Total Cell Density POL E vs SAL Males difference

Decile	POL E Males	SAL Males	Difference	CI lower	CI upper	p crit	p-value
1	3890.556	4592.76	-702.20394	-1305.4195	563.4912	9.92E-05	0.050
2	4198.377	4758.65	-560.27311	-1263.0703	1046.9528	1.28E-04	0.165
3	4541.701	4913.09	-371.38933	-1431.1522	1022.9294	2.23E-04	0.402
4	4875.572	5017.954	-142.38194	-1134.8485	1126.6137	4.46E-04	0.723
5	5198.289	5119.018	79.27151	-1152.8401	1482.5871	8.93E-04	0.878
6	5551.005	5276.107	274.89765	-1102.0542	1564.0392	2.98E-04	0.571
7	5958.77	5507.953	450.81698	-889.3568	1638.6579	1.79E-04	0.400
8	6384.625	5752.175	632.4499	-915.8302	1525.3192	1.49E-04	0.204

9	6719.294	5979.987	739.30689	-917.1017	1659.7137	1.12E-04	0.082

Supplementary Table 15. Total Cell Density POL E vs SAL Females difference

Decile	POL E Females	SAL Females	Difference	CI lower	CI upper	p crit	p-value
1	4740.61	1278.007	3462.6034	237.65558	5054.735	1.24E-05	<0.001
2	5050.65	2870.448	2180.2024	-16.12843	4808.611	1.40E-05	0.002
3	5343.483	4116.629	1226.8536	-208.66979	4457.246	1.59E-05	0.004
4	5647.352	4663.776	983.576	-246.80128	4170.709	1.86E-05	0.013
5	5938.522	4967.196	971.3257	-348.42508	2812.317	2.23E-05	0.018
6	6165.649	5246.656	918.9926	-870.52609	2246.009	2.79E-05	0.038
7	6315.302	5568.478	746.8239	-1895.1188	1546.637	3.72E-05	0.186
8	6413.841	6219.588	194.2528	-2279.906	1330.446	1.12E-04	0.803
9	6478.831	7441.747	-962.9169	-1905.2061	1109.814	5.58E-05	0.296

Supplementary Table 16. Total Cell Density POL L vs SAL Males difference

Decile	POL L Males	SAL Males	Difference	CI lower	CI upper	p crit	p-value
1	3719.626	4592.76	-873.13456	-1565.047	104.1645	0.00555556	0.019
2	4193.846	4758.65	-564.80345	-1404.595	323.8518	0.00625	0.091
3	4556.589	4913.09	-356.50166	-1210.0169	397.5971	0.00833333	0.252
4	4845.353	5017.954	-172.60043	-894.2499	566.5292	0.01666667	0.555

Decile							
5	5128.069	5119.018	9.050946	-672.8681	596.2203	0.05	0.966
6	5420.083	5276.107	143.975209	-700.5618	859.4345	0.025	0.725
7	5721.102	5507.953	213.149652	-689.2292	1347.9808	0.0125	0.538
8	6121.657	5752.175	369.481884	-575.4137	1439.9775	0.01	0.326
9	6675.606	5979.987	695.618625	-309.4015	1524.7984	0.00714286	0.104

Supplementary Table 17. Total Cell Density POL L vs SAL Females difference

Decile	POL L Females	SAL Females	Difference	CI lower	CI upper	p crit	p-value
1	3619.508	1278.007	2341.50133	-1145.09	3878.7923	0.00079365	0.058
2	4000.07	2870.448	1129.6216	-1132.916	3584.8689	0.00102041	0.306
3	4328.748	4116.629	212.11839	-1338.191	3318.5848	0.00119048	0.641
4	4689.957	4663.776	26.18074	-1150.641	2392.5997	0.00357143	0.850
5	5047.756	4967.196	80.5597	-1485.716	1504.3793	0.00238095	0.858
6	5388.548	5246.656	141.8913	-1471.263	1410.6258	0.00178571	0.817
7	5720.328	5568.478	151.84953	-1620.906	1146.9019	0.00714286	0.863
8	6017.608	6219.588	-201.98024	-2357.425	1111.491	0.00142857	0.761
9	6308.068	7441.747	-1133.6796	-2521.643	915.5942	0.00089286	0.189

Supplementary Table 18. Total Cell Density POL L vs POL E Males difference

Decile	POL L Males	POL E Males	Difference	CI lower	CI upper	p crit	p-value
1	3719.626	3890.556	-170.93062	-2012.992	988.9933	6.20E-06	0.577

2	4193.846	4198.377	-4.530336	-1340.008	1315.8678	1.40E-05	0.918
3	4556.589	4541.701	14.887671	-1829.684	1491.186	2.79E-05	0.987
4	4845.353	4875.572	-30.218496	-1521.274	1266.2713	1.86E-05	0.974
5	5128.069	5198.289	-70.220568	-1627.329	1431.4893	1.12E-05	0.886
6	5420.083	5551.005	-130.92244	-1739.814	1423.9628	9.30E-06	0.804
7	5721.102	5958.77	-237.66733	-1587.876	1667.94	7.97E-06	0.707
8	6121.657	6384.625	-262.96802	-1554.451	1560.6667	6.98E-06	0.673
9	6675.606	6719.294	-43.688261	-1783.333	1625.4563	5.58E-05	0.950

Supplementary Table 19. Total Cell Density POL L vs POL E Females difference

Decile	POL L Females	POL E Females	Difference	CI lower	CI upper	p crit	p-value
1	3619.508	4740.61	-1121.1021	-2448.207	44.29931	6.20E-06	0.005
2	4000.07	5050.65	-1050.5808	-2553.967	229.04088	6.98E-06	0.014
3	4328.748	5343.483	-1014.7353	-2429.921	252.09046	7.97E-06	0.023
4	4689.957	5647.352	-957.3952	-2450.655	472.80904	9.30E-06	0.050
5	5047.756	5938.522	-890.766	-2284.841	657.12797	1.40E-05	0.068
6	5388.548	6165.649	-777.1013	-2096.116	446.71426	1.86E-05	0.074
7	5720.328	6315.302	-594.9744	-1825.747	528.25807	1.12E-05	0.099
8	6017.608	6413.841	-396.2331	-1466.23	579.40294	2.79E-05	0.091
9	6308.068	6478.831	-170.7627	-1428.37	316.42526	5.58E-05	0.257

Supplementary Table 20. Dark Glial Cell Density POL E vs SAL Males difference

Decile	POL E Males	SAL Males	Difference	CI lower	CI upper	p crit	p-value
1	8.38E-04	0.05848903	-0.0576506	-5.546827	0.2453002	0.00555556	0.325
2	1.45E-02	0.66519951	-0.6507007	-14.568047	0.9111334	0.00277778	0.169
3	9.78E-02	2.71532356	-2.6175609	-19.498768	1.6843484	0.00185185	0.113
4	3.70E-01	6.40184165	-6.0318349	-26.918845	3.1898038	0.00138889	0.068
5	9.70E-01	11.330745	-10.360625	-36.812142	3.8026146	0.00111111	0.029
6	2.13E+00	17.4356958	-15.305585	-41.49908	3.4525547	0.00092593	0.019
7	4.36E+00	25.1249898	-20.764221	-46.360696	1.9584087	0.00079365	0.007
8	7.93E+00	35.0327005	-27.100784	-47.848487	-2.3724743	0.00069444	<0.001
9	1.16E+01	45.9910106	-34.348116	-49.747092	-3.8059681	0.00061728	0.001

Supplementary Table 21. Dark Glial Cell Density POL E vs SAL Females difference

Decile	POL E Females	SAL Females	Difference	CI lower	CI upper	p crit	p-value
1	2.67E-07	0.00012305	-1.23E-04	-0.3919406	0.0215986	6.17E-04	0.274
2	1.77E-05	0.00886431	-8.85E-03	-6.6256465	0.2175098	3.09E-04	0.248
3	4.38E-04	0.14457666	-1.44E-01	-7.2009708	0.4655823	2.06E-04	0.16
4	5.66E-03	0.948631	-9.43E-01	-10.775766	0.9544673	1.54E-04	0.102
5	4.37E-02	3.30487748	-3.26E+00	-14.76165	0.9190349	1.23E-04	0.041

6							
	2.17E-01	7.13369719	-6.92E+00	-16.266664	1.3335848	1.03E-04	0.020
7							
	7.34E-01	11.0482462	-1.03E+01	-16.838071	1.2533821	8.82E-05	0.007
8							
	1.77E+00	14.469097	-1.27E+01	-17.151448	-1.2405593	7.72E-05	<0.001
9							
	3.17E+00	16.9509635	-1.38E+01	-17.795357	-5.8959528	6.86E-05	<0.001

Supplementary Table 22. Dark Glial Cell Density POL L vs SAL Males difference

Decile	POL L Males	SAL Males	Difference	CI lower	CI upper	p crit	p-value
1	1.96E-11	0.05848903	-0.058489	-5.963521	-3.61E-06	0.00625	0.001
2	1.83E-08	0.66519951	-0.6651995	-10.885144	-3.00E-04	0.00555556	0.003
3	4.34E-06	2.71532356	-2.7153192	-17.675691	-7.66E-03	0.00714286	0.004
4	3.96E-04	6.40184165	-6.4014458	-22.330719	-2.75E-02	0.00833333	0.007
5	1.63E-02	11.330745	-11.314395	-29.669673	3.20E-01	0.01	0.014
6	3.30E-01	17.4356958	-17.105331	-36.697382	9.43E+00	0.0125	0.048
7	3.39E+00	25.1249898	-21.739506	-42.869066	2.07E+01	0.01666667	0.178
8	1.79E+01	35.0327005	-17.090667	-45.318803	3.37E+01	0.025	0.487
9	5.05E+01	45.9910106	4.50162926	-40.953521	3.58E+01	0.05	0.882

Supplementary Table 23. Dark Glial Cell Density POL L vs SAL Females difference

Decile	POL L Females	SAL Females	Difference	CI lower	CI upper	p crit	p-value
1	2.07428	0.00012305	2.074157	0.06985707	12.83886	0.00833333	<0.001

Decile							
2	5.177012	0.00886431	5.168148	0.22991869	21.28388	0.00714286	0.005
3	9.1135	0.14457666	8.968923	1.26540404	23.81753	0.0125	0.004
4	14.175393	0.948631	13.226762	1.99193799	24.78776	0.025	0.012
5	19.610566	3.30487748	16.305688	3.86350082	26.17121	0.05	0.007
6	24.351703	7.13369719	17.218006	0.68907144	28.56021	0.01666667	0.015
7	28.086174	11.0482462	17.037928	1.49294562	32.33729	0.00625	0.005
8	32.027146	14.469097	17.558049	5.29958358	35.22357	0.01	0.001
9	40.542751	16.9509635	23.591788	7.70944969	36.11253	0.00555556	<0.001

Supplementary Table 24. Dark Glial Cell Density POL L vs POL E Males difference

Decile	POL L Males	POL E Males	Difference	CI lower	CI upper	p crit	p-value
1	1.96E-11	8.38E-04	-0.0008384	-0.8403059	2.57E-04	8.57E-06	0.029
2	1.83E-08	1.45E-02	-0.0144988	-1.6214064	1.95E-02	7.62E-06	0.058
3	4.34E-06	9.78E-02	-0.0977583	-5.1346023	1.14E+00	9.80E-06	0.063
4	3.96E-04	3.70E-01	-0.369611	-6.4914696	3.03E+00	1.37E-05	0.124
5	1.63E-02	9.70E-01	-0.9537708	-10.329422	1.10E+01	1.71E-05	0.242
6	3.30E-01	2.13E+00	-1.7997457	-12.339504	3.01E+01	2.29E-05	0.491
7	3.39E+00	4.36E+00	-0.9752849	-12.008474	4.91E+01	6.86E-05	0.893
8	1.79E+01	7.93E+00	10.0101164	-13.003822	6.48E+01	3.43E-05	0.588
9	5.05E+01	1.16E+01	38.8497452	-13.378246	7.40E+01	1.14E-05	0.1285

Supplementary Table 25. Dark Glial Cell Density POL L vs POL E Females difference

Decile	POL L Females	POL E Females	Difference	CI lower	CI upper	p crit	p-value
1	2.07428	2.67E-07	2.07428	0.00506411	20.39504	1.14E-05	<0.001
2	5.177012	1.77E-05	5.176995	0.24945673	21.60263	5.72E-06	<0.001
3	9.1135	4.38E-04	9.113062	1.58581475	27.90209	3.81E-06	<0.001
4	14.175393	5.66E-03	14.169732	3.19963492	29.24835	2.86E-06	<0.001
5	19.610566	4.37E-02	19.566873	4.35266336	30.91274	2.29E-06	<0.001
6	24.351703	2.17E-01	24.134496	6.61876696	38.23302	1.91E-06	<0.001
7	28.086174	7.34E-01	27.35232	8.18192076	43.75649	1.63E-06	<0.001
8	32.027146	1.77E+00	30.256825	12.0509654	49.0833	1.43E-06	<0.001
9	40.542751	3.17E+00	37.370737	19.7019744	49.96782	1.27E-06	<0.001

Supplementary Table 26. Dark Neuron Density POL E vs SAL Males difference

Decile	POL E Males	SAL Males	Difference	CI lower	CI upper	p crit	p-value
1	7.95E-07	0.01338757	-0.0133868	-1.656836	0.01105213	0.00555556	0.087
2	5.23E-05	0.21362932	-0.2135771	-6.000844	0.15721379	0.00277778	0.053

Decile							
3	1.28E-03	1.12113573	-1.1198526	-6.815987	0.7895302	0.00185185	0.06
4	1.63E-02	2.94651928	-2.9302176	-11.610068	0.92498822	0.00138889	0.068
5	1.22E-01	4.95042709	-4.828388	-21.334763	2.10711202	0.00111111	0.037
6	5.76E-01	6.83510042	-6.2595173	-26.797794	2.27674329	0.00092593	0.024
7	1.78E+00	10.0453915	-8.2656551	-32.964434	1.62940028	0.00079365	0.014
8	3.71E+00	17.3375998	-13.625091	-33.323013	0.00326122	0.00069444	0.003
9	5.41E+00	29.53897	-24.128274	-36.842743	-0.3306611	0.00061728	0.001

Supplementary Table 27. Dark Neuron Density POL E vs SAL Females difference

Decile	POL E Females	SAL Females	Difference	CI lower	CI upper	p crit	p-value
1	1.11E-05	1.63E-09	1.11E-05	-0.0093335	0.1719587	8.82E-05	0.317
2	4.38E-04	9.10E-07	4.37E-04	-0.0929765	0.5793316	1.03E-04	0.357
3	6.47E-03	1.04E-04	6.36E-03	-0.6910162	1.7857513	1.23E-04	0.483
4	4.97E-02	3.99E-03	4.57E-02	-2.3319386	1.9669653	1.54E-04	0.609
5	2.28E-01	6.49E-02	1.63E-01	-3.6154481	2.3426595	3.09E-04	0.744
6	6.77E-01	5.07E-01	1.69E-01	-6.5124234	2.710026	6.17E-04	0.963
7	1.40E+00	2.10E+00	-7.04E-01	-8.1366172	2.6683049	2.06E-04	0.668
8	2.20E+00	5.11E+00	-2.91E+00	-10.199566	2.603619	7.72E-05	0.242
9	2.79E+00	8.71E+00	-5.92E+00	-11.88331	1.2480937	6.86E-05	0.019

Supplementary Table 28. Dark Neuron Density POL L vs SAL Males difference

Decile	POL L Males	SAL Males	Difference	CI lower	CI upper	p crit	p-value
1	1.80E-12	0.01338757	-0.0133876	-3.619961	-1.57E-07	0.01	0.001
2	1.69E-09	0.21362932	-0.2136293	-5.673354	-6.32E-06	0.00555556	0.002
3	4.07E-07	1.12113573	-1.1211353	-6.429677	-4.73E-05	0.00714286	0.006
4	3.84E-05	2.94651928	-2.9464809	-9.063155	-1.52E-03	0.00833333	0.006
5	1.71E-03	4.95042709	-4.9487126	-14.725415	-1.21E-02	0.00625	0.007
6	4.04E-02	6.83510042	-6.7947181	-21.616597	-3.87E-01	0.0125	0.008
7	5.32E-01	10.0453915	-9.5133155	-27.359307	3.69E+00	0.01666667	0.053
8	3.85E+00	17.3375998	-13.483337	-31.442905	5.99E+00	0.025	0.132
9	1.40E+01	29.53897	-15.579848	-31.565599	6.54E+00	0.05	0.177

Supplementary Table 29. Dark Neuron Density POL L vs SAL Females difference

Decile	POL L Females	SAL Females	Difference	CI lower	CI upper	p crit	p-value
1	1.32E-04	1.63E-09	0.00013228	-0.0002651	0.3096555	0.00625	0.136
2	5.25E-03	9.10E-07	0.00524855	-0.0151697	1.7952565	0.01	0.159
3	7.19E-02	1.04E-04	0.07180021	-0.1671349	5.7450419	0.00833333	0.198
4	4.91E-01	3.99E-03	0.4866887	-0.6373064	8.9532267	0.0125	0.206
5	1.94E+00	6.49E-02	1.87750889	-0.6823101	8.6566387	0.05	0.206
6	4.86E+00	5.07E-01	4.35560012	-1.9815046	10.6758468	0.025	0.190
7	8.32E+00	2.10E+00	6.21590361	-4.3655219	12.1094618	0.01666667	0.202

8	1.11E+01	5.11E+00	5.97530913	-6.2419107	14.2211203	0.00714286	0.129
9	1.43E+01	8.71E+00	5.60573642	-5.7310481	14.0907629	0.00555556	0.118

Supplementary Table 30. Dark Neuron Density POL L vs POL E Males difference

Decile	POL L Males	POL E Males	Difference	CI lower	CI upper	p crit	p-value
1	1.80E-12	7.95E-07	-7.95E-07	-0.0928224	3.90E-05	9.80E-06	0.236
2	1.69E-09	5.23E-05	-5.23E-05	-0.7129505	1.96E-03	8.57E-06	0.3375
3	4.07E-07	1.28E-03	-1.28E-03	-2.2161713	1.00E-01	1.14E-05	0.314
4	3.84E-05	1.63E-02	-1.63E-02	-3.156025	7.54E-01	1.37E-05	0.357
5	1.71E-03	1.22E-01	-1.20E-01	-5.8132401	3.74E+00	1.71E-05	0.442
6	4.04E-02	5.76E-01	-5.35E-01	-5.6806593	1.05E+01	2.29E-05	0.551
7	5.32E-01	1.78E+00	-1.25E+00	-5.7970739	1.56E+01	3.43E-05	0.700
8	3.85E+00	3.71E+00	1.42E-01	-5.9888879	1.89E+01	6.86E-05	0.937
9	1.40E+01	5.41E+00	8.55E+00	-5.996452	2.20E+01	7.62E-06	0.2705

Supplementary Table 31. Dark Neuron Density POL L vs POL E Females difference

Decile	POL L Females	POL E Females	Difference	CI lower	CI upper	p crit	p-value
1	1.32E-04	1.11E-05	1.21E-04	-0.3745774	1.694192	7.62E-06	0.663
2	5.25E-03	4.38E-04	4.81E-03	-0.5793341	2.711498	3.81E-06	0.556
3	7.19E-02	6.47E-03	6.54E-02	-1.8740314	8.01383	2.54E-06	0.510

4	4.91E-01	4.97E-02	4.41E-01	-1.836736	10.340975	1.91E-06	0.459
5	1.94E+00	2.28E-01	1.71E+00	-2.3481622	11.328048	1.52E-06	0.331
6	4.86E+00	6.77E-01	4.19E+00	-2.3535711	12.426122	1.27E-06	0.183
7	8.32E+00	1.40E+00	6.92E+00	-2.7252112	13.838796	1.09E-06	0.084
8	1.11E+01	2.20E+00	8.88E+00	-2.5829455	16.457213	9.53E-07	0.015
9	1.43E+01	2.79E+00	1.15E+01	-2.4035657	17.33054	8.47E-07	0.006

Supplementary Table 32. Apoptotic Cell Density POL E vs SAL Males difference

Decile	POL E Males	SAL Males	Difference	CI lower	CI upper	p crit	p-value
1	5.75E-06	5.23E-08	5.70E-06	-0.200738	0.08309331	1.32E-04	0.536
2	2.32E-04	7.00E-06	2.25E-04	-0.1985263	0.52506395	1.13E-04	0.529
3	3.57E-03	2.89E-04	3.29E-03	-0.7255787	1.17588146	1.59E-04	0.534
4	2.94E-02	5.34E-03	2.41E-02	-1.3666148	2.05924476	2.65E-04	0.644
5	1.51E-01	5.09E-02	1.00E-01	-1.8282966	3.81782995	7.94E-04	0.715
6	5.41E-01	2.70E-01	2.71E-01	-2.5794743	5.62691195	3.97E-04	0.670
7	1.47E+00	8.33E-01	6.41E-01	-3.0073851	6.51577473	1.98E-04	0.645
8	3.27E+00	1.65E+00	1.63E+00	-3.0865078	6.58912238	9.92E-05	0.443
9	5.94E+00	2.73E+00	3.21E+00	-3.799139	7.4130836	8.82E-05	0.279

Supplementary Table 33. Apoptotic Cell Density POL E vs SAL Females difference

Decile	POL E Females	SAL Females	Difference	CI lower	CI upper	p crit	p-value
1	0.06077409	2.19E-08	0.06077406	-5.89E-05	2.003436	9.80E-06	0.001
2	0.32815958	7.40E-06	0.32815218	-4.91E-02	2.109396	1.10E-05	0.005
3	0.80343016	5.01E-04	0.80292952	-5.25E-01	2.564185	1.26E-05	0.011
4	1.33231219	1.12E-02	1.3210813	-6.16E-01	2.998568	1.47E-05	0.011
5	1.81378203	1.05E-01	1.70897342	-6.95E-01	4.165108	1.76E-05	0.020
6	2.28556947	4.73E-01	1.81283812	-1.20E+00	4.706171	2.20E-05	0.029
7	2.8913935	1.17E+00	1.72067703	-1.03E+00	4.483863	2.94E-05	0.044
8	3.74678314	1.86E+00	1.884857	-1.68E+00	4.577465	4.41E-05	0.064
9	4.6245176	2.88E+00	1.74712351	-1.97E+00	4.056836	8.82E-05	0.168

Supplementary Table 34. Apoptotic Density POL L vs SAL Males difference

Decile	POL L Males	SAL Males	Difference	CI lower	CI upper	p crit	p-value
1	3.28E-09	5.23E-08	-4.90E-08	-0.0044623	0.00031029	0.00625	0.787
2	9.07E-07	7.00E-06	-6.10E-06	-0.071187	0.0107912	0.00833333	0.794
3	6.39E-05	2.89E-04	-2.25E-04	-0.0538405	0.03489436	0.05	0.844
4	1.75E-03	5.34E-03	-3.58E-03	-0.5735756	0.21625223	0.01666667	0.823
5	2.22E-02	5.09E-02	-2.87E-02	-1.5118993	0.77329311	0.0125	0.830
6	1.45E-01	2.70E-01	-1.25E-01	-1.6858897	1.0409658	0.025	0.878

7						
	5.32E-01	8.33E-01	-3.01E-01	-2.3399603	1.59191438	0.01	0.763
8						
	1.25E+00	1.65E+00	-3.94E-01	-2.8467626	2.50316015	0.00555556	0.718
9						
	2.41E+00	2.73E+00	-3.13E-01	-2.8257589	3.08950572	0.00714286	0.766

Supplementary Table 35. Apoptotic Cell Density POL L vs SAL Females difference

Decile	POL L Females	SAL Females	Difference	CI lower	CI upper	p crit	p-value
1	3.56E-09	2.19E-08	-1.84E-08	-0.0012782	0.0005374	0.00714286	0.809
2	5.54E-07	7.40E-06	-6.84E-06	-0.0885355	0.02471114	0.00357143	0.683
3	2.84E-05	5.01E-04	-4.72E-04	-0.461889	0.15471194	0.00238095	0.601
4	6.82E-04	1.12E-02	-1.05E-02	-0.9482446	0.42394535	0.00178571	0.551
5	8.92E-03	1.05E-01	-9.59E-02	-1.7132934	0.96299854	0.00142857	0.494
6	6.83E-02	4.73E-01	-4.04E-01	-2.2593282	1.17609701	0.00119048	0.399
7	3.19E-01	1.17E+00	-8.52E-01	-2.4625073	1.39261982	0.00102041	0.334
8	9.27E-01	1.86E+00	-9.35E-01	-3.5729426	1.37456302	0.00089286	0.252
9	1.68E+00	2.88E+00	-1.20E+00	-4.5062637	0.9738635	0.00079365	0.084

Supplementary Table 36. Apoptotic Cell Density POL L vs POL E Males difference

Decile	POL L Males	POL E Males	Difference	CI lower	CI upper	p crit	p-value
1	3.28E-09	5.75E-06	-5.75E-06	-0.1954404	0.00559849	1.10E-05	0.338
2	9.07E-07	2.32E-04	-2.31E-04	-2.004306	0.03225349	1.47E-05	0.373

3	6.39E-05	3.57E-03	-3.51E-03	-2.3097949	0.37140159	1.76E-05	0.412
4	1.75E-03	2.94E-02	-2.77E-02	-3.1251772	0.86786421	2.94E-05	0.505
5	2.22E-02	1.51E-01	-1.29E-01	-3.8677683	0.96845351	8.82E-05	0.528
6	1.45E-01	5.41E-01	-3.96E-01	-6.1913104	1.35167046	4.41E-05	0.548
7	5.32E-01	1.47E+00	-9.41E-01	-7.5731441	1.73926306	2.20E-05	0.449
8	1.25E+00	3.27E+00	-2.02E+00	-7.7372568	2.34876766	1.26E-05	0.344
9	2.41E+00	5.94E+00	-3.53E+00	-7.6425497	3.35488955	9.80E-06	0.248

Supplementary Table 37. Apoptotic Cell Density POL L vs POL E Females difference

Decile	POL L Females	POL E Females	Difference	CI lower	CI upper	p crit	p-value
1	3.56E-09	0.06077409	-0.0607741	-2.016653	2.90E-03	1.09E-06	0.002
2	5.54E-07	0.32815958	-0.328159	-2.439921	-2.59E-05	1.96E-06	<0.001
3	2.84E-05	0.80343016	-0.8034018	-2.835063	9.02E-03	4.90E-06	0.002
4	6.82E-04	1.33231219	-1.3316299	-3.844567	-6.84E-03	3.27E-06	0.001
5	8.92E-03	1.81378203	-1.8048616	-4.413767	3.18E-01	1.63E-06	0.005
6	6.83E-02	2.28556947	-2.217292	-4.535999	1.47E-01	2.45E-06	0.003
7	3.19E-01	2.8913935	-2.5723796	-4.869634	8.07E-01	1.40E-06	0.005
8	9.27E-01	3.74678314	-2.8199705	-4.978101	-1.08E-01	9.80E-06	0.001
9	1.68E+00	4.6245176	-2.9462621	-4.993	-8.56E-02	1.22E-06	<0.001

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Maternal Immune Activation Model Reporting Guidelines Checklist

ARRIVE Reporting Guideline & Recommendation	Arrive Item	MIA Model Specific Reporting Recommendation *Please complete this chart for each point outlined below. If not applicable, write N/A*

Study design
➢Overview of immune activation issues
 	
For each experiment, give brief details of the study design including:
    a. The number of experimental and control groups.
    b. Any steps taken to minimize the effects of subjective bias when allocating animals to treatment (e.g. randomization procedure) and when assessing results (e.g. if done, describe who was blinded and when).
    c. The experimental unit (e.g. a single animal, group or cage of animals).
 	
A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.
	6	MIA Specific Reporting:
		a. General need for improved reporting in MIA model methods + reporting pilot data
		    o Details on pilot data:

Experimental procedures
➢Compounds
➢Validation measures
 	
For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example:
    a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s).
    b. When (e.g. time of day).
    c. Where (e.g. home cage, laboratory, water maze).
    d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).
	7	Provide details of:
		a. Compounds – source, vehicle, preparation/storage, administration route, volume administered, whether anesthetics were used at time of immune challenge.
		    o Name of compound:
		    o Catalogue number:
		    o Lot number:
		    o Vehicle control used:
		    o Route of administration:
		    o Volume administered:
		    o Storage conditions:
		    o Anesthetic (type, dose, duration) used:
		
		b. Housing variables at injection - temperature of room at injection time, cage change at time of injection or not
		    o Light cycle of animal housing room:
		    o Time of day of injection:
		    o Room temperature at injection time:
		    o Did a cage change occur at time of injection:

		c. Validation of immune activation – behavior, physiological indices and/or cytokine data, including pilot dosing data
		    ○ Method used to verify immune activation:
		
		d. Validation of gestational timing – vaginal plug, estrous cycle, weight gain
		    ○ Method of validating gestational timing:
		
		Additional comments:

Experimental animals
➢Species/strain/vendor
 	
a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range).
b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.
	8	Provide details of:
		a. Species – considerations for appropriate species (mouse, rat, non human primate, other)
		    ○ Species:
		
		b. Strain – variability in strain can influence model
		    ○ Strain:
		
		c. Maternal/Offspring Physiological Variables at time of immune challenge – age, body weight
		    ○ Maternal Age at challenge:
		    ○ Maternal Body weight:
		    ○ Offspring Age at challenge:
		    ○ Offspring Sex:
		    ○ Offspring Body weight:
		
		d. Vendor – even within the same strain, vendor can influence endpoints
		    ○ Vendor:
		    ○ Location of Vendor:
		    ○ Room/area where animals originated from:

		Additional Comments:

Housing and husbandry
➢Cage, ventilation, bedding, enrichment
 	
Provide details of:
a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish).
b. Husbandry conditions (e.g. breeding program, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment).
c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.
	9	Provide details of:
		a. Caging systems
		    ○ *At breeding*
		        Material of cage:
		        Cage dimensions:
		    ○ *After parturition*
		        Material of cage:
		        Cage dimensions:
		    ○ *At weaning*
		        Material of cage:
		        Cage dimensions:
		
		b. Animal Holding room
		    ○ Temperature in room:
		    ○ Humidity in room:
		    ○ Ventilation system:
		    ○ Specific pathogen free [SPF]:
		    ○ Are males & females housed in the same or separate rooms:
		
		c. Bedding exchanges/bedding type
		    ○ Type of cage bedding used:
		    ○ Frequency of cage changes per week
		        during gestation:
		        during neonatal period:
		        following weaning:
		
		d. Breeding - bred on site or timed pregnant, how many different sires (are the same fathers breeding with both experimental and control dams)
		    Breeding location:

- Gestational age at shipping:
- Biological age of dams (if not listed in Section 8c):
- Number of Dams bred:
- How many times have dams been mated previously:
- How many times did the dams mate and not become pregnant:
- Are the dams primiparous or multiparous?
- What was the frequency of maternal handling during the gestational/neonatal period (e.g. cage cleanings, weighing, blood collection manipulations):
- Biological age of sires:
- Number of sires bred:
- How many times have sires been mated previously:
- How many times did the sires mate successfully (e.g. mating resulted in pregnancy, full term birth):
- If bred previously, what was the interval between mating times:
- Are sires matched to experimental and control dams:
- Describe the mating design (1:1, 1:2 etc):

e. Social enrichment – number of cage companions
- Number of cage companions prior to breeding:
- Gestational age when dam separated for parturition:
- Number of cage companions at weaning:

f. Physical enrichment – describe enrichment devices, and when enrichment is in the cage (removed when pups born? Or present throughout study), does the enrichment type change? How frequently?
- Describe what type of enrichment devices (and how many) are included in cage/housing room:

		o Does enrichment type/access change across study?
		o If so, when does enrichment type/access change (e.g. enrichment removed prior to parturition and replaced in late neonatal period):
		Additional Comments:
Sample size ➢Litter versus offspring a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.	10	Provide details of: a. Maternal N vs offspring N o What is the total number of dams/litters included in the study: o What is the total number of offspring per litter included the study: b. Litter size and sex distribution o What size was each litter maintained at: o What age did culling take place at: o How many males and females were maintained in each litter: c. Cross fostering o Did cross fostering occur: o If so, at what age did cross fostering occur: Additional Comments:

Allocating animals to experimental groups
 	
a. Give full details of how animals were allocated to experimental groups, including randomization or matching if done.
b. Describe the order in which the animals in the different experimental groups were treated and assessed.
	11	a. How many offspring per litter were used in each measure:
		
		b. Randomization/Matching procedures
		    o What procedures were used to assign animals to groups:
		
		c. Sex as a biological variable (behavioral and physiological outcomes)
		    o Were both males and females evaluated in each behavioral and physiological outcome:
		
		Additional Comments:

Experimental outcomes
➢Behavioral testing
➢Physiological endpoints
 	
Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioral changes).
	12	a. Maternal behavior and pup interactions
		    o If maternal care was evaluated, were there differences following immunogen challenge (if so, please briefly describe):
		
		b. Age(s) of offspring at behavioral testing/physiological evaluation endpoints:
		
		c. Order of testing (e.g. behavioral test order)
		    o Were animals evaluated in a counter-balanced order in terms of:
		        presentation of tests to each animal:
		        order of experimental/control groups run through each test:
		    o What was the inter-test interval if a single animal underwent a battery of tests:

		Additional Comments:
Statistical methods a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	13	a. Unit of analysis for each data set o Is the unit (n) of each analysis based on number of litters, or number of animals used per group:
Other Disclosures		Please make note of any other extraneous variables that you would like to report (e.g. fire alarms, construction, temporary relocations, other variables that you think we should be considering in our studies etc.):

The recommended use of this reporting form is to fill it out and include it as supplemental material for each of your laboratory's research publications. If there are difficulties utilizing/adapting this fillable form, please contact one of the corresponding authors to request a copy. The authors give permission for this table to be edited for use in reporting on other animal models (e.g. postnatal immune challenge models, early life stress models) as appropriate.

Kentner AC, Bilbo AD, Brown AS, Hsiao EY, McAllister AK, Meyer U, Pearce BD, Pletnikov MV, Yolken RH, Bauman MD. (2018). Maternal immune activation: reporting guidelines to improve the rigor, reproducibility, and transparency of the model. Neuropsychopharmacology, https://doi.org/10.1038/s41386-018-0185-7.