Supplementary Table 1. MIA model reporting details

ARRIVE Reporting Guideline & Recommendation	Arrive Item	MIA Model Specific Reporting Recommendation
Study Design	6	MIA Specific Reporting
Overview of Immune Activation issues a. Number of Control groups: 1 (saline treated animals) Number of Experimental groups: 9 (20, 30,40mg/kg, low, medium and high BIR) b. Steps taken to minimize subjective bias: Dams were randomized into cages upon receipt, offspring were randomized to dosage vs control group but were not randomized to BIR as this was dependent on a fixed trait in their mothers. All scoring was done by researchers blinded to the animals' treatment and BIR. c. The experimental unit: litters with male and female offspring		a. Details on Pilot data: prior to study several pilots were conducted to determine protocol for serum collection, BIR determination and determination of the E12.5 time point by weight gain of the animals during pregnancy. Serum collection was done at 2.5 hours and 4 hours, with the highest IL -6 peak seen at 2.5 hours rather than the previous reports of a 4-hour IL-6 peak (Figure 2). BIR was determined by poly(I:C) lot, and low, medium and high delineations were generated using the quartiles of the normal distribution (low = lowest quartile, medium = middle two quartiles, high = highest quartile). Piloted gestational dissections during gestation in which researchers estimated the age of the pups based on morphology determined that the E12.5 time point on average occurred during the 9.5-10.5 weight gain from time of mating range.
Experimental Procedures	7	Details:
Compounds used: a. Saline control during gestation (0.9% NaCl physiological endotoxin free saline) b. Baseline IL-6 response (BIR) tested with Invivogen Poly(I:C), gestational treatment used Sigma Poly (I:C) c. Procedures ▪ Drug administration: animals were tested for their BIR at 7 weeks of age, one week after arrival to the facility. They were given an IP injection of 4.2mg/kg ± 0.2mg/kg Invivogen using a 1cc insulin syringe (BD Micro-Fine™ IV Insulin Syringes) ▪ All injections occurred in the morning between 7-10am ▪ Injections were done in ventilation hoods in the home vivarium of the animals. ▪ Pregnant animals were given IP injections as previously described (Garay et al., 2013) using the doses of Sigma poly(I:C) as indicated in the manuscript.		a. Compounds: ▪ Saline vehicle and control o Name: sterile 0.9% saline o Catalogue Number: 7647-14-5 o Lot number: N/A o Route of Administration: intraperitoneal (IP) injection o Volume Administered: 5µL/gram of body weight o Storage Conditions: stored at 21 ± 1°C o No anesthetic used ▪ Invivogen Poly (I:C) o Name: high molecular weight (HMW) poly (I:C) dsRNA o Catalogue Number: #tlrl-pic o Lot number: pic-37-8, pic-39-7 o Route of Administration: IP injection o Volume Administered: 5µL/gram of body weight o Storage Conditions: aliquoted and stored at 4°C o No anesthetic used ▪ Sigma Poly (I:C) o Name: mixed molecular weight (MMW) poly (I:C) dsRNA o Catalogue Number: #P0913 o Lot number: 016M1451V #38, #39, #67, #69, #70, #71, #73 o Route of Administration: IP injection o Volume Administered: 5µL/gram of body weight o Storage Conditions: aliquoted and stored at 4°C o No anesthetic used b. Housing variables: ▪ Light cycle: 12 hours of light 7am-7pm ▪ Mean time of day at injection: 8:50am ▪ Room temperature at injection time: 21 ± 1°C
Experimental Animals	8	Details:
Species/strain/vendor: Mouse, C57BL/6N from Charles River (CR; Kingston, NY), Taconic (TAC; Hudson, NY), and C57BL/6J from Jackson (JAX; Sacramento, CA) a. Maternal details: virgin female animals were ordered at 6 weeks ± 3 days and requested to be cage mates born on the same day from the same location. BIR was determined 1 week after arrival at 7 weeks ± 3 days. Mating was set up at 8 weeks ± 3 days. b. Offspring of dams ordered from Charles River/Taconic/JAX were used in this study after being exposed to either vehicle control or Poly(I:C) during pregnancy at E12.5. Behavioral phenotyping was done at P60 while biochemical assessment was done immediately postnatally.		a. Species: Mouse b. Strain/Vendor: C57BL/6N from Charles River (CR; Kingston, NY), Taconic (TAC; Hudson, NY), and C57BL/6J from Jackson (JAX; Sacramento, CA) c. Maternal/offspring physiological variables at time of immune challenge ▪ Maternal Age at Challenge: 7 weeks ▪ Maternal Body Weight: 26.3-34.4 grams ▪ Offspring Age at Challenge: E12.5 ▪ Offspring Sex: Males and Females ▪ Offspring Body Weight: N/A d. Vendor ▪ C57BL/6N Charles River (CR; Kingston, NY), ▪ C57BL/6N Taconic (TAC; Hudson, NY) ▪ C57BL/6J Jackson (JAX; Sacramento, CA)

c. Animals were not genetically modified and were all wild-type. Their health/immune status was normal, they were drug and test naïve and had no previous procedures.		
Housing and Husbandry	9	Details:
Cage, ventilation, bedding, enrichment: a. Housing Type: Animals were housed at maximum capacity of 4/cage in Tecniplast Sealsafe® individually ventilated cages (IVC). Cages had a 1" ± 0.5" layer of corn cob bedding, enviro-dri rodent bedding and single nestlets. Males were provided huts to prevent aggression, and if aggression was observed were given tubes in addition to huts. b. Husbandry conditions: breeding occurred at 8 weeks ± 3 days, animals were housed at a maximum of 4/cage on a 12-hour light cycle 7am-7pm, animals were given ad libitum access to food (Envigo Teklad) and water (stored in bottles), the average temperature of the vivarium was 21 ± 1°C. c. Animals received daily wellness checks to assess for intra-cage aggression, potential injuries or sickness and overall health. Cage changes occurred every 2 weeks and were carried out after behavioral assessment.		a. Caging systems ▪ At breeding, after parturition and at weaning: o Make: Tecniplast Sealsafe® o Material of Cage: transparent polysulfone plastic with microbiological filter (Virus filtration efficiency ≥ 99.999987%, bacteria filtration efficiency ≥ 99.9999937%), nylon gasket and latches, non-invasive rack nozzles to avoid cross-contamination and DOP tested HEPA filters o Cage Dimensions: 67.42in² ▪ Animal Holding Room o Temperature in room: 21 ± 1°C o Ventilation system: Sealsafe® o Specific pathogen free (SPF): o Males and females are housed in same room, separate cages ▪ Bedding exchanges/bedding type o Type of cage bedding used: corncob o Frequency of cage changes per week ✦ During gestation: biweekly ✦ During neonatal period: biweekly ✦ Following weaning: biweekly ▪ Breeding: on site o Dams' age at shipping: 6 weeks ± 3 days o Biological age of dam: see section 8c o Number of dams bred: 210 o Number of times dams been mated previously: 0, dams were virgin females o Number of times dams mated and didn't become pregnant: N/A o Sires matched to experimental and control dams o Mating design: 1:1 ▪ Social Enrichment: o Number of cage companions prior to breeding ✦ Dams: 1-3 virgin female age-matched companions ✦ Sires: singly housed o Gestational age when dam separated for parturition: E12.5 o Number of cage companions at weaning: offspring had 1-3 same-sex sibling cage companions, no singly housed animals were used ▪ Physical Enrichment: all Tecniplast cages contain metal food hoppers for climbing. Cages also included nestlets and enviro-dri. Male cage, parturition cages and sire cages contained plastic huts. Male offspring with observed aggression were given plastic tubes in addition to huts. All enrichment was changed biweekly.
Sample Size	10	Details:
Litter vs. Offspring a. Total number of animals in each behavioral experiment ▪ Charles River: n = 419 ▪ Taconic: n = 160 b. Number of animals in each experimental group (sorted by sex, BIR and dosage) ▪ Charles River o Saline males: n = 37 o Low 20mg/kg males: n = 13 o Medium 20mg/kg males: n = 22 o High 20mg/kg males: n = 21 o Low 30mg/kg males: n = 16 o Medium 30mg/kg males: n = 29 o High 30mg/kg males: n = 19 o Low 40mg/kg males: n = 9 o Medium 40mg/kg males: n = 21 o High 40mg/kg males: n = 14		a. Maternal N vs. Offspring N ▪ Total number of dams/litters used to generate behavioral data: o Dams: 334 o Litters: 183 ▪ Number of offspring per litter included in the study: 2-4 animals/sex/litter ▪ Total number of dams/litters included in the biochemical assays: o Dams: 8 saline, 8 low, 6 medium, 6 high o Litters: 8 saline, 8 low, 6 medium, 6 high b. Litter size and sex distribution ▪ Litters were maintained at 2-4 animals per sex per litter ▪ Animals were culled after P120 ▪ 2-4 males and females per litter were maintained c. Cross fostering: N/A

○ Saline females: n = 37 ○ Low 20mg/kg females: n = 22 ○ Medium 20mg/kg females: n = 21 ○ High 20mg/kg females: n = 24 ○ Low 30mg/kg females: n = 17 ○ Medium 30mg/kg females: n = 20 ○ High 30mg/kg females: n= 18 ○ Low 40mg/kg females: n= 16 ○ Medium 40mg/kg females: n = 23 ○ High 40mg/kg females: n = 20 ▪ Taconic ○ Saline males: n = 28 ○ Low 30mg/kg males: n = 16 ○ Medium 30mg/kg males: n = 16 ○ High 30mg/kg males: n = 18 ○ Saline females: n = 33 ○ Low 30mg/kg females: n = 13 ○ Medium 30mg/kg females: n = 18 ○ High 30mg/kg females: n = 18 c. Number of independent replications of each experiment: N/A		
Allocating animals to experimental groups	11	Details:
a. Allocation and randomization of animals: Dams were randomized to cages upon arrival. After BIR measurement animals were allocated to control and dosage groups randomly, but BIR was a fixed and not randomized trait. b. All offspring were scored by researchers blinded to their experimental treatment. Offspring for biochemistry were dissected without behavioral assessment. Offspring were handled by experimenters for 3 minutes/day for 3 days prior to videotaped behavioral assessment of grooming and rearing at P60.		a. Offspring per litter used in each measure: ▪ Biochem: 2 males/litter ▪ Behavior: 2-4/sex/litter b. Randomization/matching procedures ▪ Offspring were assigned to BIR group per their mothers' readout, dosage and treatment was randomized within BIR c. Sex as a biological variable ▪ Both males and females were evaluated in each behavioral outcome ▪ Males only were evaluated for biochemical outcomes
Experimental Outcomes	12	Details:
a. Behavioral testing: grooming/rearing/freezing assessed as described in text b. Physiological endpoints: offspring tissue tested for MHCI, MEF2A, STAT3, TH, VAMP2, PSD95; dam's fecal samples tested via qPCR for SFB analysis		a. Maternal behavior and pup interactions: N/A b. Age of offspring at behavioral testing: P60 c. Order of behavioral testing: ▪ All animals underwent assessment of grooming and rearing at P60
Statistical Methods	13	Details:
a. Details of statistical methods used for each analysis: Mixed Model nested one- way ANOVA, followed by Tukey's post hoc test. This test considers a subset of data within the data, which produces a more accurate picture of our results. In this case, the comparative dataset is control versus treatment (litter average), with a subset of measured animals per litter. This allows for the analysis to include the variance we found within litters, as well as adjust for unequal sample sizes per litter average. b. Unit of analysis for each dataset: The unit used for analysis (n) is the number of litters, where the litter average is the descriptive statistic with corrections made by the mixed model nested ANOVA (GraphPad Prism v7) to account for unequal variance between litter size and/or conditions. c. Methods used to assess whether the data met the assumptions of statistical approach: Assumption of normality: GraphPad Prism (v7) was used to assess normality of control and treatment populations. Assumption of homogeneity of variance: This was not met for any treatment groups across litter size (to determine litter average), so a mixed model approach nested ANOVA was adopted. As per GraphPad: "Prism uses a mixed model approach when		a. Unit of analysis for each data set ▪ The unit (n) of each analysis is based on the number of litters as well as the number of animals used per group as the statistical analyses were done using a nested ANOVA correcting for unequal variance between conditions.

repeated measures designs are unbalanced (have unequal sample sizes). This is an improvement on the Satterthwaite approximation." Satterthwaite approximation being a similar adjustment that can be applied to nested ANOVAs when this assumption is not met. Assumption of sample independence: Dams and pups were randomized as described above.		