Supporting Information

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SI Methods

Mice. C57BL/6N mice (Charles River) were housed under specific pathogen-free conditions in the California Institute of Technology's Broad animal facility. Mice were mated overnight and the presence of a vaginal plug on the following morning was noted as embryonic day (E) 0.5. All experiments were performed under the approval of the California Institute of Technology Institutional Animal Care and Use Committee.

Spleen and Mesenteric Lymph Node Suspensions. Spleens and mesenteric lymph nodes (MLNs) were harvested, washed in complete RPMI media on ice, and forced through 40-μm cell strainers (BD Biosciences). In some trials, spleens from the same treatment group were pooled in groups of three to facilitate accommodating a large number of samples, while preserving cell viability. Single-cell suspensions were subjected to RBC lysis (Sigma Aldrich). $CD4^+$ T cells were isolated by negative selection using a $CD4^+$ Isolation kit (Miltenyi Biotec). Cell counts were performed on a hemocytometer, and 10^6 cells were aliquoted for each $CD4^+$ stimulation reaction.

Flow Cytometry. For extracellular staining, cells were treated with anti-mouse CD16/CD32 Fc block (eBioscience) before staining with subsets of these antibody conjugates: Ter119-PerCP-Cy5.5, CD4-FITC, CD8-FITC, Gr-1-APC, B220-FITC, CD11b-APC (eBioscience), NK1.1-PE (Biolegend).

Regulatory T-Cell Suppression Assay. For regulatory T-cell (Treg) suspression assay, 10^5 $CD4^+CD25^-$ T-responder cells were cultured in complete RPMI with 2×10^4 irradiated antigen-presenting cells and 2.5 μg/mL anti-CD3, with $CD4^+CD25^+$ Tregs at a ratio to T-responder cells of 1:1, 1:2, and 1:4, according to methods from (1). After 2 d of culture, suspensions were stained with CD4-APC antibody and assessed by flow cytometry.

Fetal Liver Suspensions. Fetal livers were harvested from E13.5 and E15.5 embryos and washed in PBS. Six to seven fetal livers from a single litter were pooled, forced through a 40-μm cell strainer, and washed with Iscove's modified Dulbecco's medium (IMDM) + 2% (vol/vol) FBS (StemCell Technologies). Cells were counted using a hemocytometer, and 2×10^5 cells used per methylcellulose solution.

Donor Cell Harvest for Bone Marrow Transplantation. Four adult saline offspring (two male, two female) and four adult poly(I:C) offspring (two male, two female) were selected from an independent cohort of behaviorally tested maternal immune activation (MIA) and control mice, and designated as bone marrow (BM) donors. Donor adult saline and poly(I:C) mice were killed by CO_2 gas, and BM was harvested by flushing femurs and tibias with DMEM (Thermo Scientific). BM suspensions from each treatment group were pooled, subjected to RBC lysis (Sigma Aldrich), filtered through a 40-μm cell strainer, and stored on ice.

Behavioral Testing. For prepulse inhibition (PPI), mice were acclimated to an SR-LAB testing chamber (SD Instruments) for 5 min, presented with six, 120-dB pulses of white noise (startle stimulus), and then subjected to 14 randomized blocks of either no startle, startle stimulus only, 5-dB prepulse + startle, or 15-dB prepulse + startle. The startle response was recorded by a piezoelectric sensor, and PPI defined as: (startle stimulus only – 5 or 15 dB prepulse + startle)/startle stimulus only × 100.

For open-field testing, mice were allowed to explore a 50 × 50-cm white Plexiglas box for 10 min. An overhead video camera was used to record the session, and Ethovision software (Noldus Information Technology) used to analyze the distance traveled, and the number of entries and duration of time spent in the center arena (central square, 17 × 17 cm).

For marble-burying testing, mice were acclimated for 10 min to a new testing cage containing Aspen pine bedding (NEPCO), 3-cm deep. The mice were briefly placed in a fresh holding cage while 18 navy blue marbles (15-mm diameter, washed with 70% ethanol and mixed in Aspen pine bedding) were laid in a 6 × 3 pattern in the testing cage. Mice were placed back to the testing cage, and the number of buried marbles (>50% covered with bedding) was scored after a 10-min trial.

For social interaction testing, mice were first habituated for 10 min to a 60 × 40-cm Plexiglas box with three equally-sized chambers, with each of the two side chambers containing an empty Plexiglas cylinder. During a second 10-min trial, the mouse was placed in the center chamber and given the choice to interact with an unfamiliar, same-sex mouse that was placed in one of the side chamber cylinders, or with a nonsocial object (novel green, sticky ball toy), placed in the other side chamber cylinder. In the third and final 10-min trial, social preference was measured by replacing the nonsocial object with a new, unfamiliar, same-sex mouse. In this trial, the mouse was given the choice to interact with the now familiar mouse (from trial 2) or the new unfamiliar mouse. The cylinders holding the stimulus mice or the toy have small holes (~10 mm diameter) over the entire surface area to permit direct interaction. Mice to be tested were prehabituated to the Plexiglas cylinders and three-chambered box for 20 min each day for 3 d before testing. All equipment was cleaned with 70% ethanol and Process NPD (STERIS Life Sciences) before and after testing. The side chamber used for placement of the social object or the nonsocial object, and the familiar mouse or the unfamiliar mouse was alternated in each trial to prevent bias. The duration of time spent and number of entries into each chamber were recorded using an overhead video camera and analyzed with Ethovision software. Equivalent levels of chamber duration and chamber entries during the habituation phase were used to ensure that the mice did not exhibit chamber bias. "Social preference, chamber duration" is measured by: (duration in the novel mouse chamber – duration in the familiar mouse chamber). "Social preference, chamber frequency" is measured by: number of entries into the novel mouse chamber – number of entries into the familiar mouse chamber. For post-BM transplant behavior testing, different stimulus (familiar and unfamiliar) mice were used to prevent potential confounding effects of social memory.

1. Collison LW, Vignali DA (2011) In vitro Treg suppression assays. *Methods in molecular biology* 707:21–37.

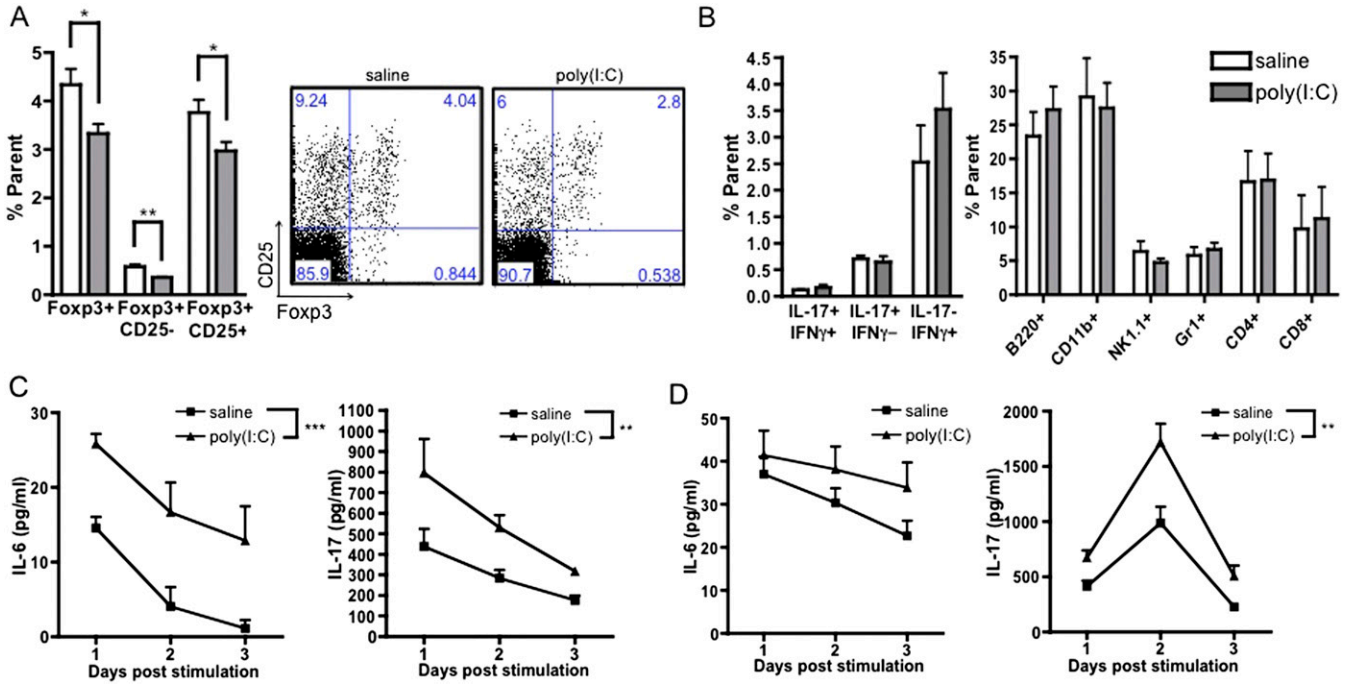

Fig. S1. Some of the immune changes observed in spleens of MIA offspring are recapitulated in the MLN. (*A*) Consistent with what is observed in the spleen, MLNs from poly(I:C) offspring exhibit decreased levels of total $CD4^+$ $Foxp3^+$, $CD4^+$ $Foxp3^+$ $CD25^-$, and $CD4^+$ $Foxp3^+$ $CD25^+$ T cells, but no significant difference in levels of $CD4^+$ $TCR\beta^+$ IL-17^+ IFN-γ^+ $Th17^+$ Th1 cells or $CD4^+$ $TCR\beta^+$ IL-17+ IFN-γ^- Th17 cells (n = 5, where each sample represents a pool of three spleens). (*B*) There are no significant differences between MLNs from adult poly(I:C) versus saline offspring in the distribution of major leukocyte classes [n = 8 saline, 9 poly(I:C)]. (*C*) Consistent with what is observed in the spleen, $CD4^+$ T cells from the MLNs from 15-wk-old poly(I:C) offspring produce increased levels of IL-6 and IL-17 at 1, 2, and 3 d after phorbol 12-myristate 13-acetate (PMA)/ionomycin stimulation (n = 4, where each represents a pool MLNs from three animals). (*D*) This hyperresponsive phenotype of MLN $CD4^+$ T cells is maintained in 1-y-old poly(I:C) versus saline mice (n = 11–16). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. All panels represent one representative experiment of at least two separate trials.

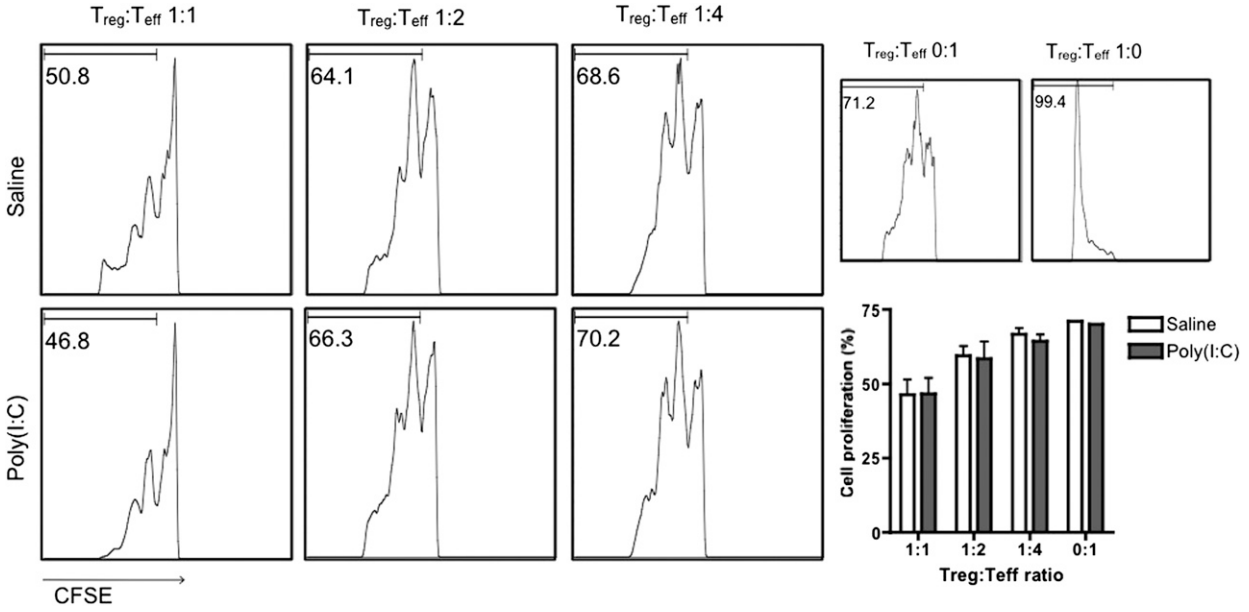

Fig. S2. There is no significant difference in the suppression of $CD4^+CD25^-$ T-cell (effector T cells, Teff) proliferation by splenic Tregs from adult saline versus poly(I:C) offspring. Similar levels of Teff proliferation are observed after 48 h of coculture of Tregs with Teffs at ratios of 1:1, 1:2, and 1:4 (n = 4).

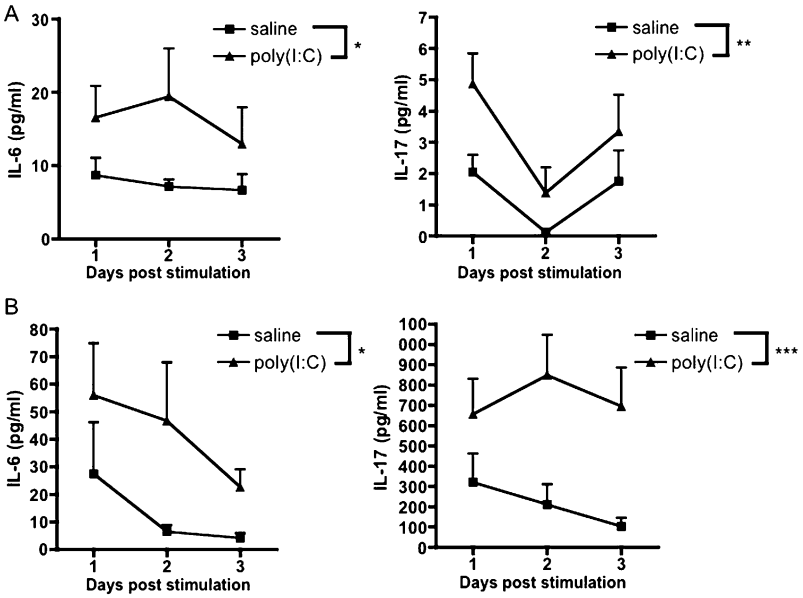

Fig. S3. Hyperresponsiveness of CD4$^+$ T cells from MIA offspring is established early in development and maintained through adulthood. CD4$^+$ T cells from the spleens of 3-wk-old MIA offspring (*A*) and 1-y-old MIA offspring (*B*) secrete elevated levels of IL-6 (*Left*) and IL-17 (*Right*) in response to PMA/ionomycin stimulation (n = 5–7). *P < 0.05, **P < 0.01, ***P < 0.001. All panels represent one representative experiment of at least two separate trials.

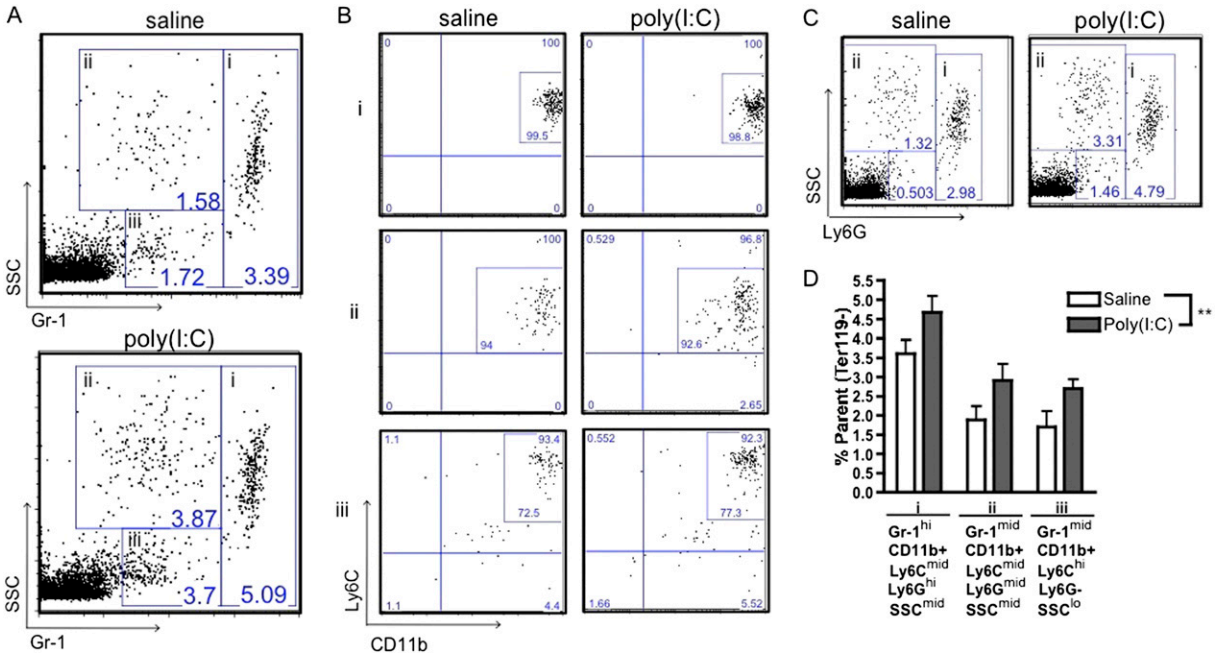

Fig. S4. Increases in both neutrophilic and monocytic Gr-1$^+$ CD11b$^+$ cells constitute the MIA-associated increase in total Gr-1$^+$ cells. (*A*) Three populations of Gr-1$^+$ cells are resolved based on Gr-1 intensity and granularity (side scatter, SSC). (*B*) Further stratification by CD11b and Ly6C intensity demonstrates that all three populations are CD11b$^+$ and Ly6C+ (high or mid). (*C*) Assessment for Ly6G intensity reveals monocytic Gr-1$^+$ cells [Ly6G$^-$, population (iii)] and neutrophilic Gr-1$^+$ cells [Ly6G high or mid, populations (i) and (ii)]. (*D*) Adult poly(I:C) offspring exhibit mild increases in all three populations of Gr-1$^+$ cells identified: (i) Gr-1^{hi} CD11b$^+$ Ly6C^{mid} Ly6G^{hi} SSC^{mid}, (ii) Gr-1^{mid} CD11b$^+$ Ly6C^{mid} Ly6G^{mid} SSC^{mid}, and (iii) Gr-1^{mid} CD11b$^+$ Ly6C^{hi} Ly6G$^-$ SSC^{lo}, which result in a statistically significant difference based on treatment group (n = 5). **P < 0.01.

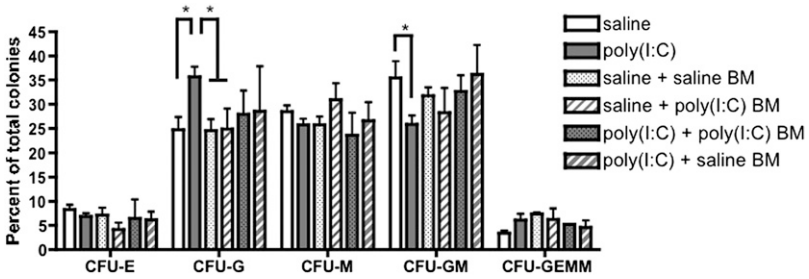

Fig. S5. There is no significant difference between unmanipulated saline mice and BM-transplanted mice in the lineage potential of BM HSCs and progenitors differentiated in vitro. Transplant of poly(I:C) or saline BM into irradiated poly(I:C) offspring corrects the abnormal lineage differentiation phenotype associated with unmanipulated poly(I:C) offspring. Similarly, transplant of poly(I:C) BM into irradiated saline offspring does not transfer the preferential skewing toward CFU-G (granulocyte) colonies that is observed in unmanipulated poly(I:C) offspring (n = 3). *P < 0.05.

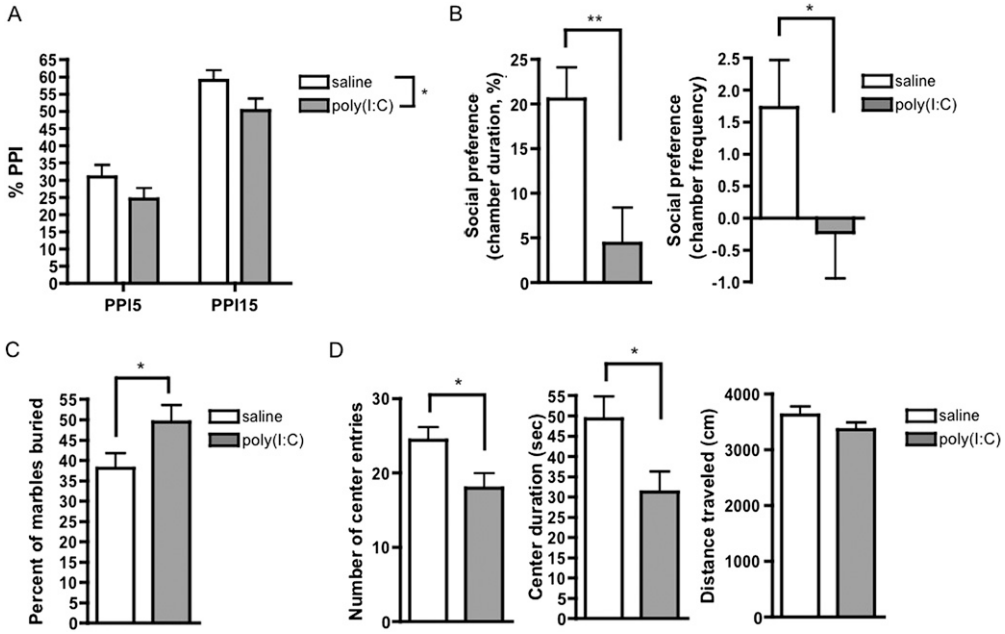

Fig. S6. BM transplant recipients are validated to be behaviorally abnormal in PPI, social preference, repetitive marble burying, and open-field exploration. (*A*) MIA offspring display significantly decreased PPI when prepulses are administered at 5 dB and 15 dB above background (PPI5 and PPI15, respectively). (*B*) Offspring of poly(I:C)-injected mothers exhibit decreased social preference for a novel mouse over a familiar mouse, as measured by differences in chamber duration (*Left*) and chamber frequency (*Right*). (*C*) MIA offspring bury significantly greater numbers of marbles compared with saline controls, pre-BM transplant. (*D*) Poly(I:C) offspring exhibit increased anxiety in the open field compared with saline controls, as measured by decreased entries into and duration in the center of the arena, and no significant difference in overall locomotion as denoted by total distance traveled (n = 20–21). *P < 0.05, **P < 0.01. All panels represent one representative experiment of at least two separate trials. BM transplant data were acquired from one large experiment.

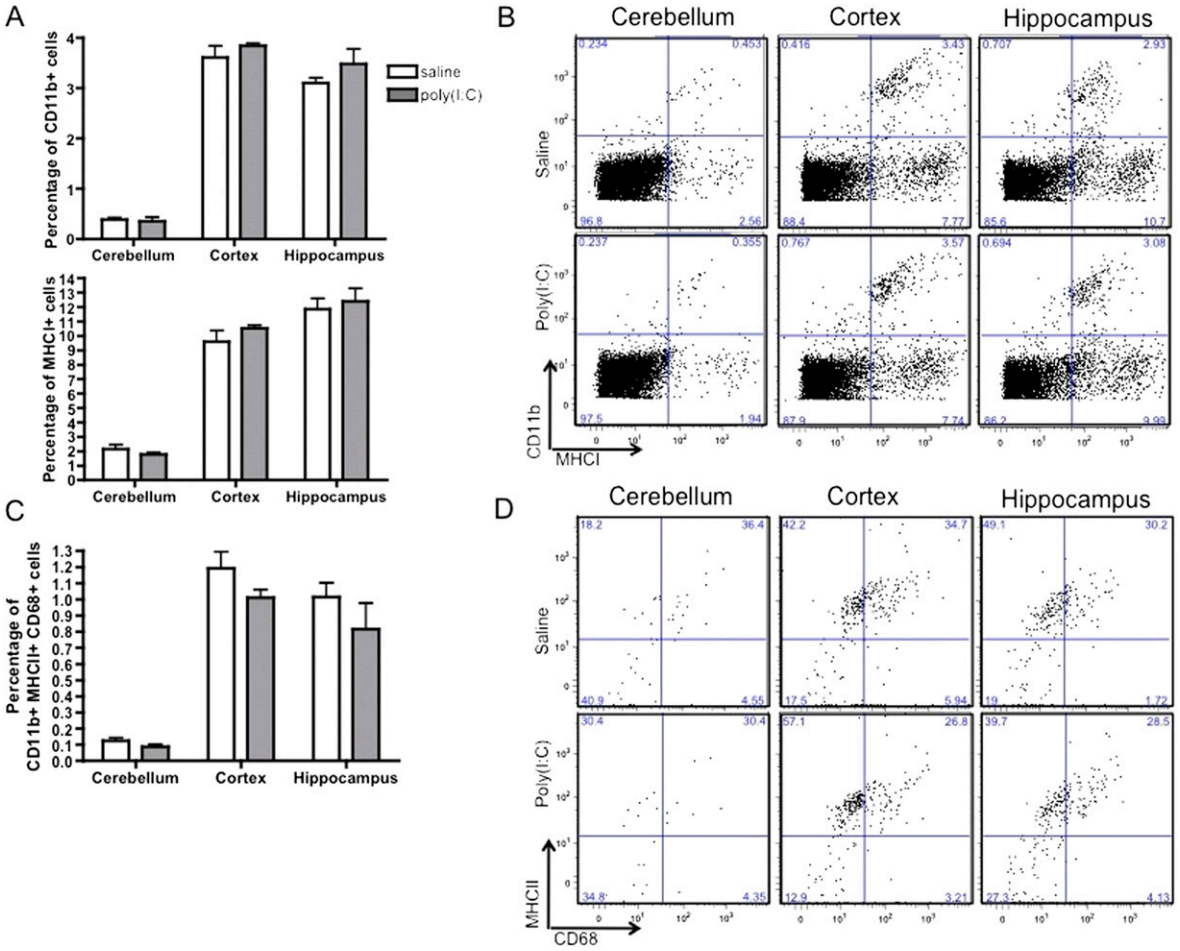

Fig. S7. MIA does not affect levels of activated or total CD11b$^+$ microglia/macrophages in the brains of adult offspring. (*A*) There is no significant difference in levels of total CD11b$^+$ cells or MHCI$^+$ cells in the cerebellum, cortex, or hippocampus of adult poly(I:C) versus saline mice. (*B*) Representative flow cytometry plots of the separation of brain CD11b$^+$ and MHCI$^+$ cells. (*C*) There is no significant difference in the percentage of CD11b$^+$ MHCII$^+$ CD68$^+$ cells from the cerebellum, cortex, or hippocampus of adult poly(I:C) versus saline mice. (*D*) Representative flow cytometry plots of the separation of brain MHCII$^+$ and CD68$^+$ cells. (n = 4 for each brain region).

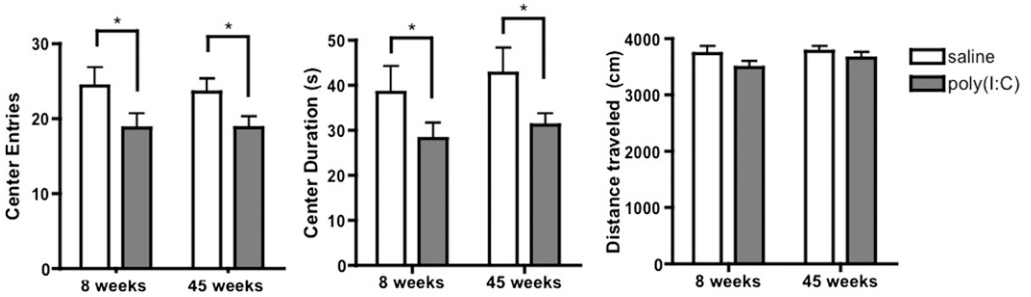

Fig. S8. MIA offspring retested for anxiety in the open field at 9 mo of age retain behavioral deficits of similar intensity to those seen at 2 mo of age. This result applies to the number of entries into the center of the open field (*Left*) and duration of time spent in the center of the open field (*Center*). No significant difference is observed between saline and poly(I:C) offspring in total distance traveled in the open field arenas (*Right*), as is typically observed in the MIA model (n = 17–19). *$P < 0.05$

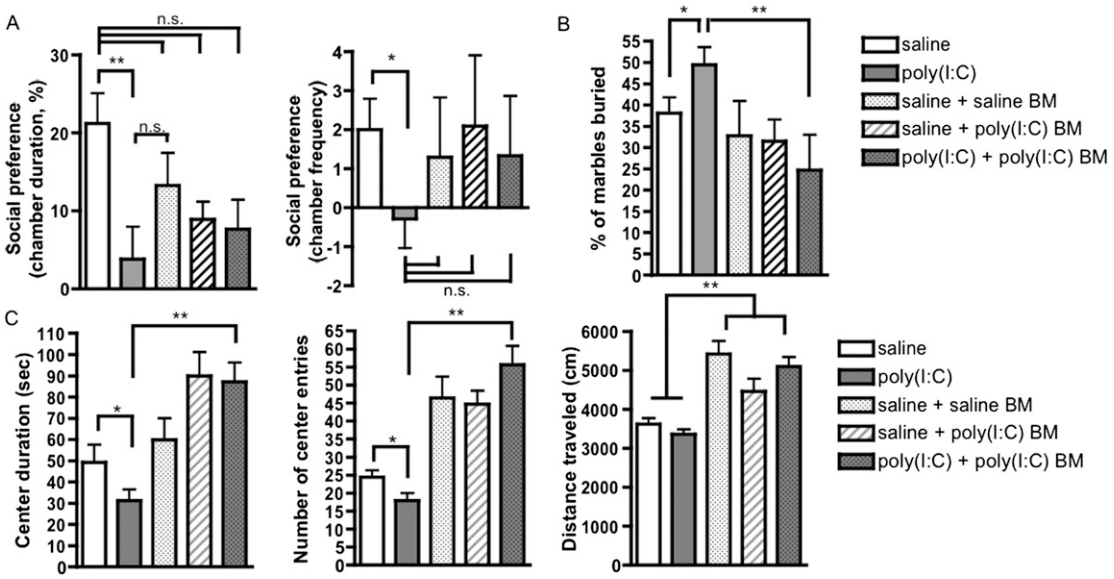

Fig. S9. The abnormal behaviors of MIA offspring are not maintained after irradiation and BM transplant. (*A*) Saline and poly(I:C) offspring irradiated and transplanted with poly(I:C) BM exhibit no difference in social preference for a novel mouse over a familiar mouse, as indicated by chamber duration (*Left*) and chamber frequency (*Right*). (*B*) MIA and control offspring irradiated and transplanted with poly(I:C) BM exhibit levels of repetitive marble burying that are comparable to those observed in transplanted control and untransplanted saline offspring. (*C*) There is no significant difference in level of anxiety in the open field between saline and poly(I:C) offspring irradiated and transplanted with saline or poly(I:C) BM, as measured by duration spent in the center of the open arena (*Left*) and number of entries into the center arena (*Center*). However, irradiated and BM-transplanted mice exhibit increased overall activity in the open field compared with untreated mice, as seen in elevated total distance traveled (*Right*). [n = 11–16 animals for saline and poly(I:C) groups, 4–5 animals for BM-transplant groups]. *$P < 0.05$, **$P < 0.01$, n.s., not significant. BM-transplant data were acquired from one large experiment.