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Supplemental Information

Maternal Inflammation Contributes to Brain Overgrowth and Autism-Associated Behaviors through Altered Redox Signaling in Stem and Progenitor Cells

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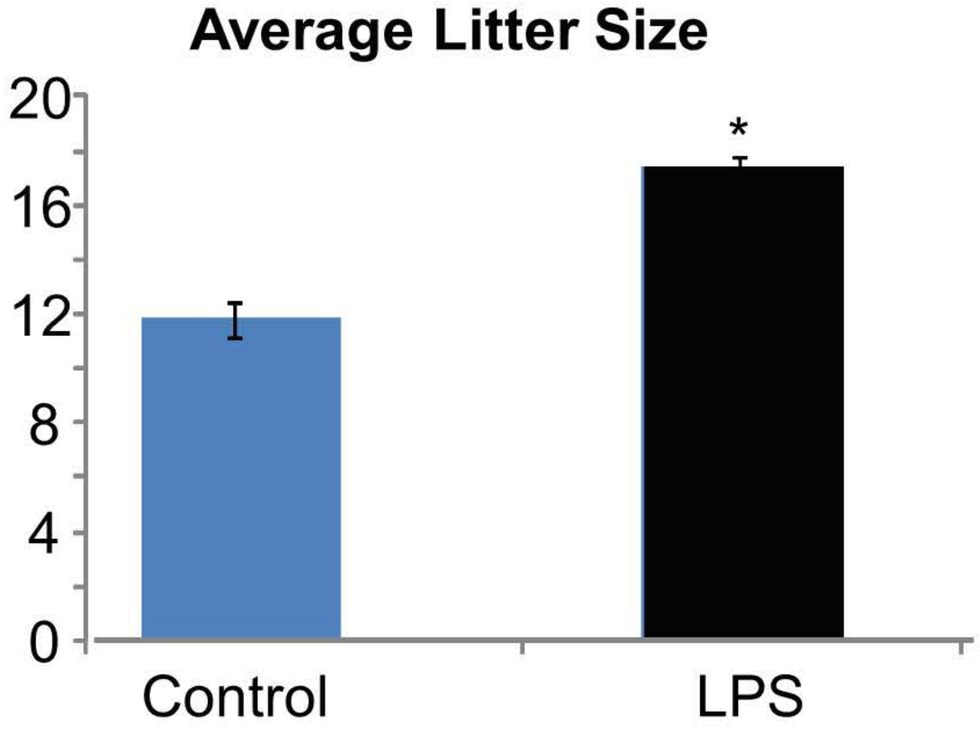
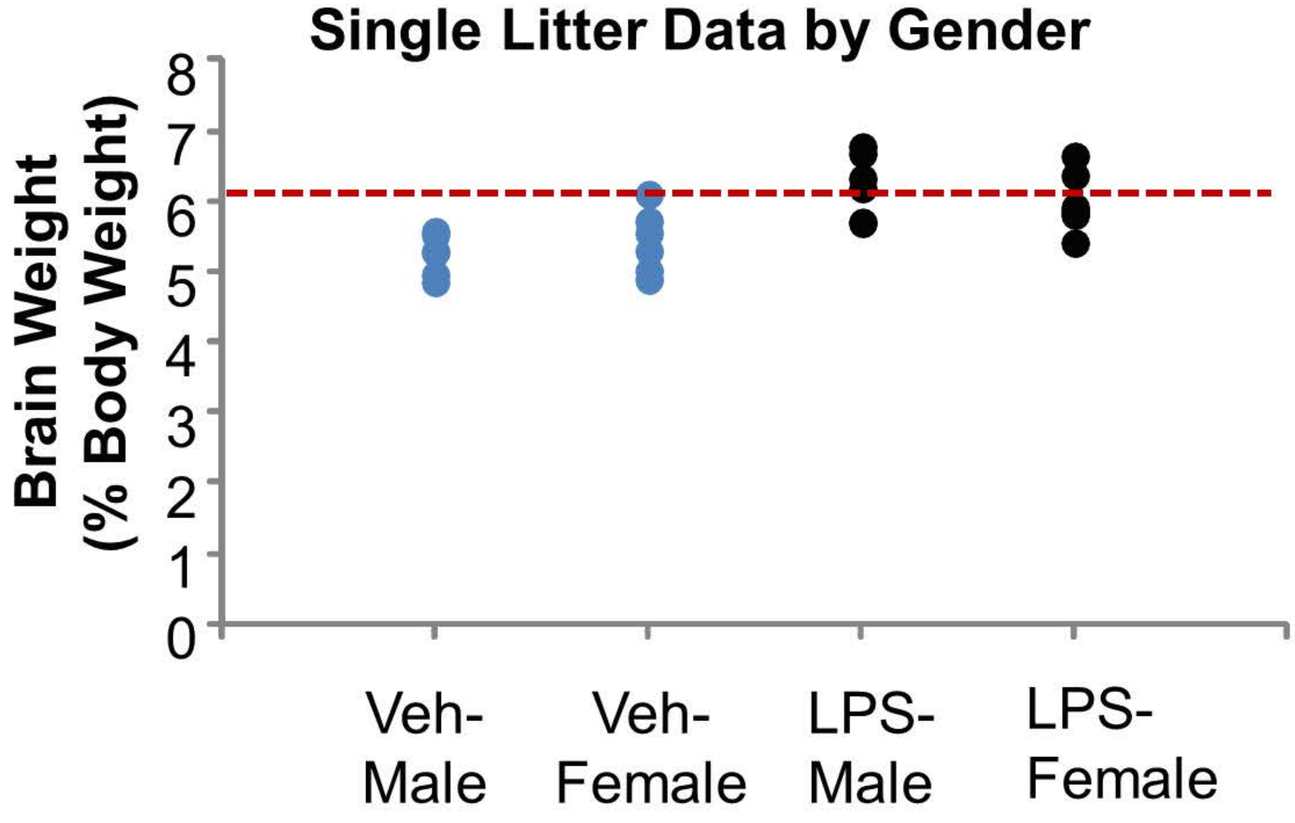
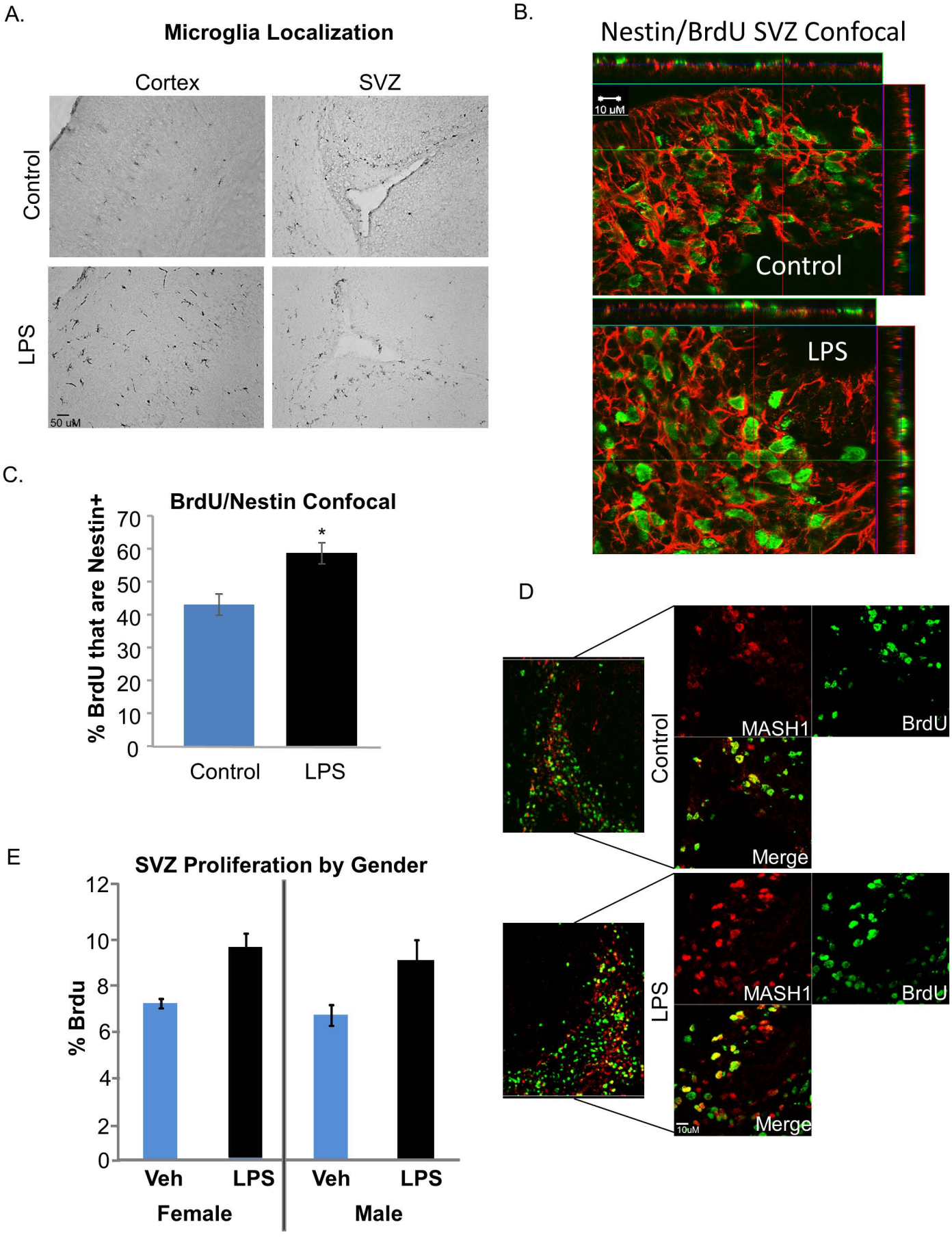
A.

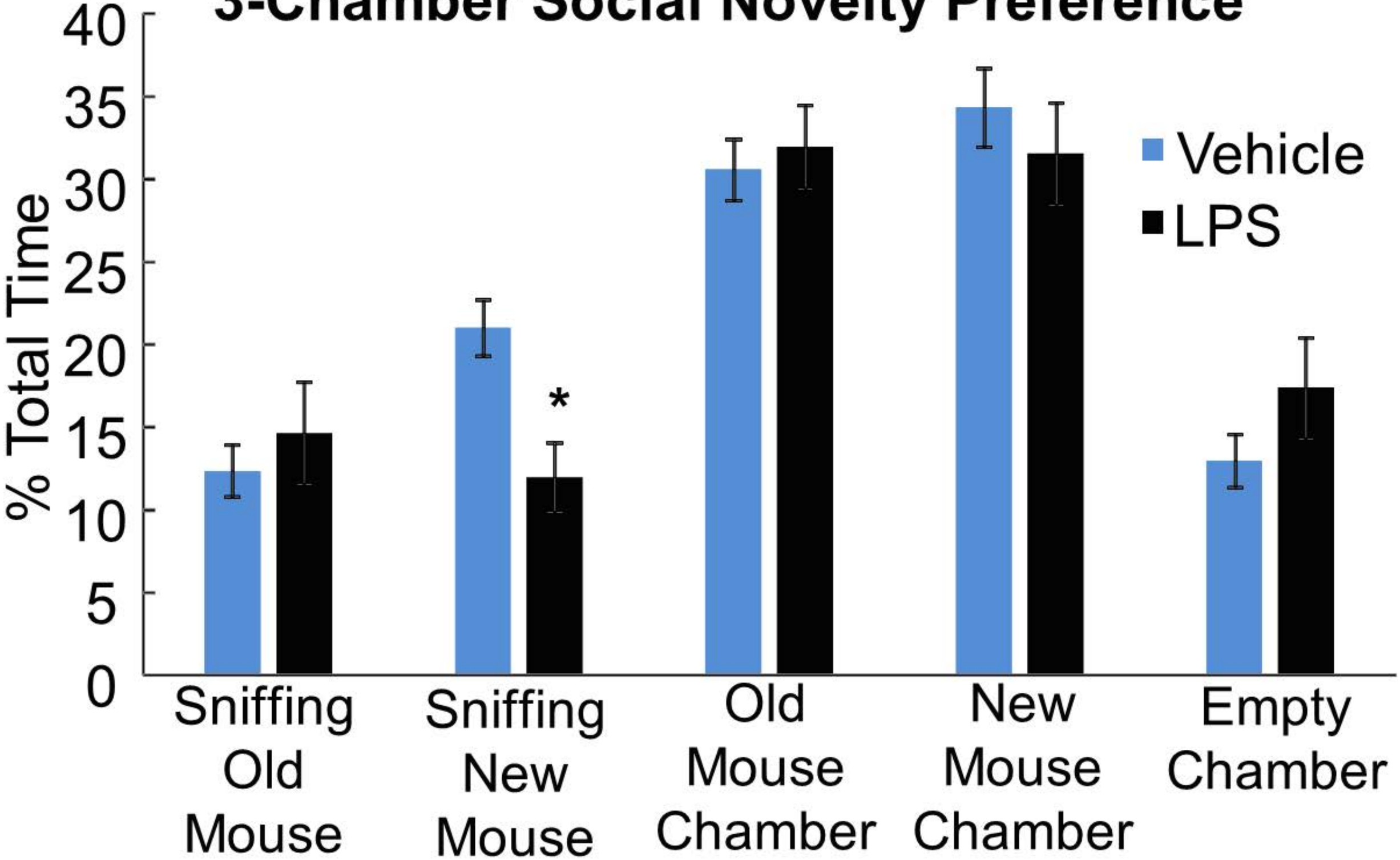

Average Litter Size
20
16
12
8
4
0
*
Control
LPS

B.

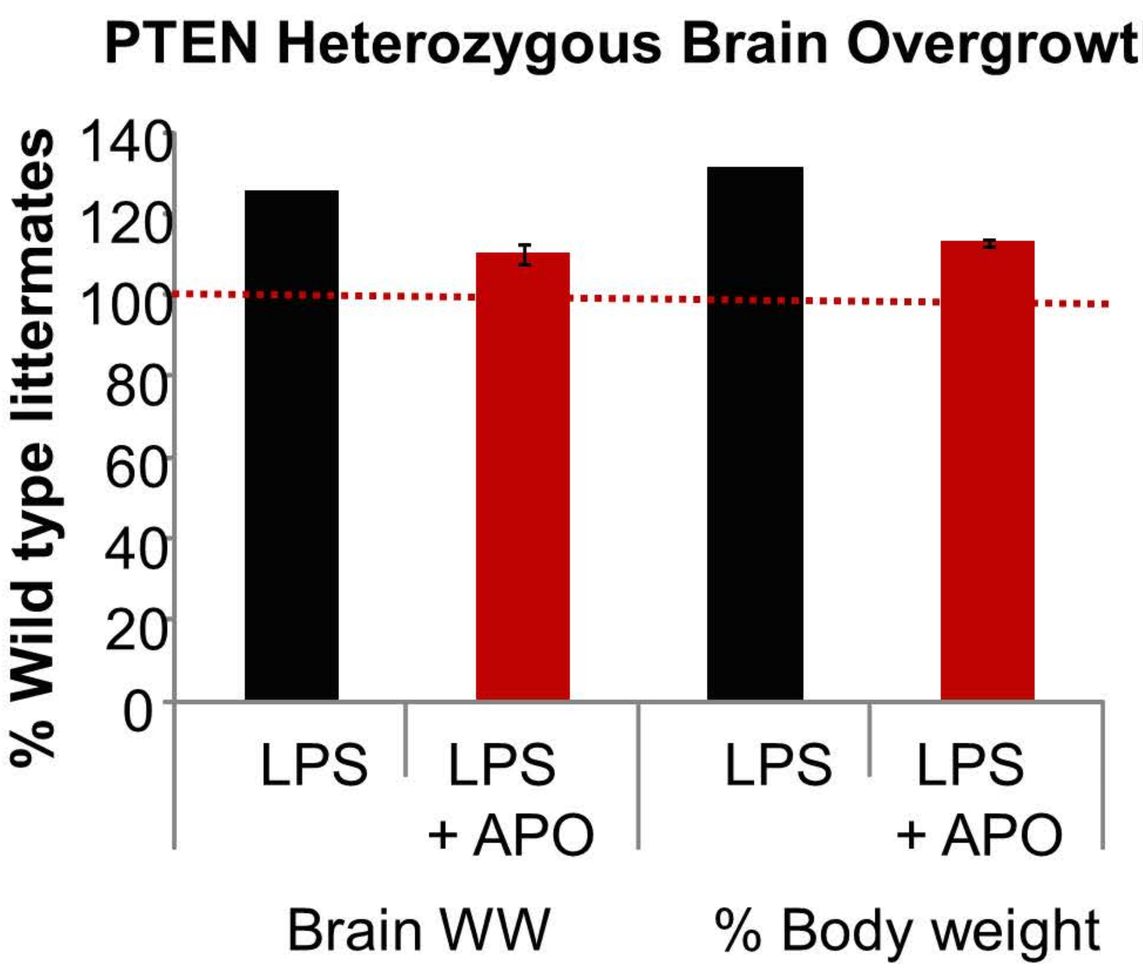

Single Litter Data by Gender
Brain Weight (% Body Weight)
8
7
6
5
4
3
2
1
0
Veh-Male
Veh-Female
LPS-Male
LPS-Female


A.
Microglia Localization
Cortex
SVZ
Control
LPS
50 uM
B.
Nestin/BrdU SVZ Confocal
10 uM
Control
LPS
C.
BrdU/Nestin Confocal
% BrdU that are Nestin+
70
60
50
40
30
20
10
0
*
Control
LPS
D
Control
MASH1
BrdU
Merge
LPS
MASH1
BrdU
Merge
10uM
E
SVZ Proliferation by Gender
% Brdu
12
10
8
6
4
2
0
Veh
LPS
Veh
LPS
Female
Male

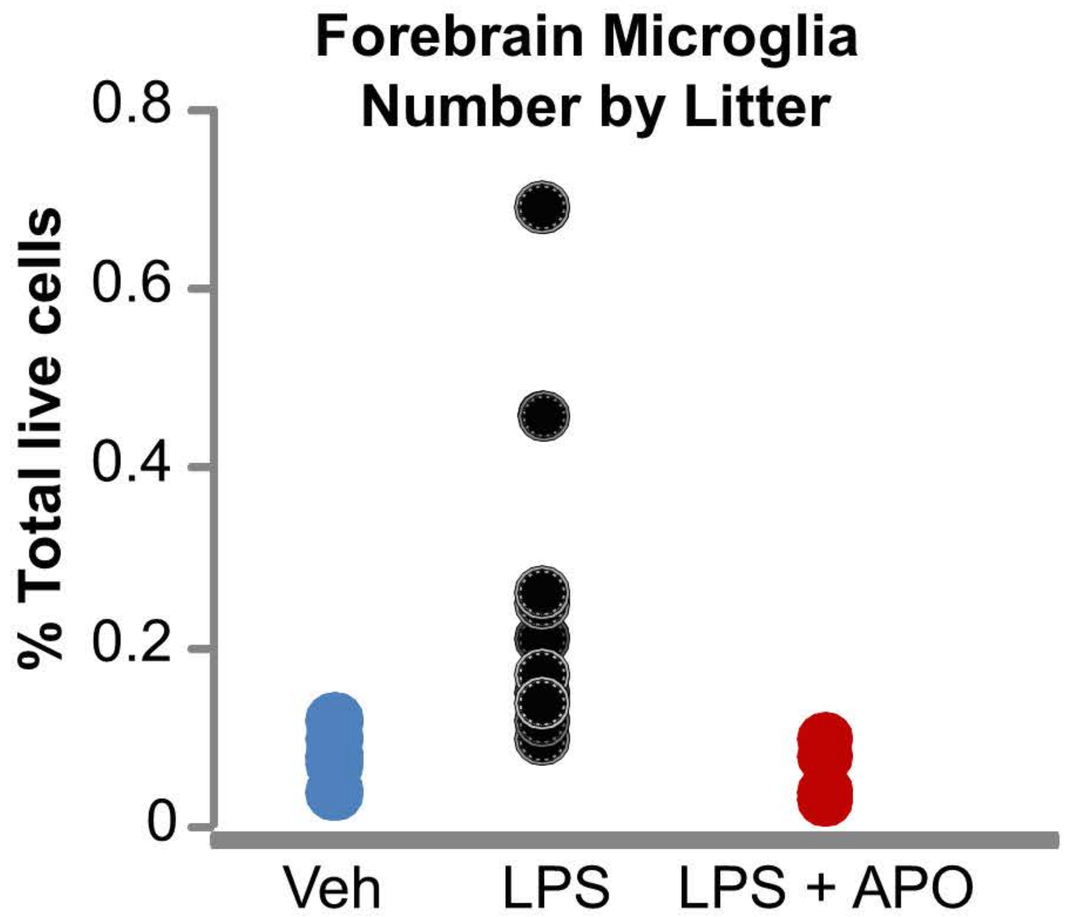
3-Chamber Social Novelty Preference



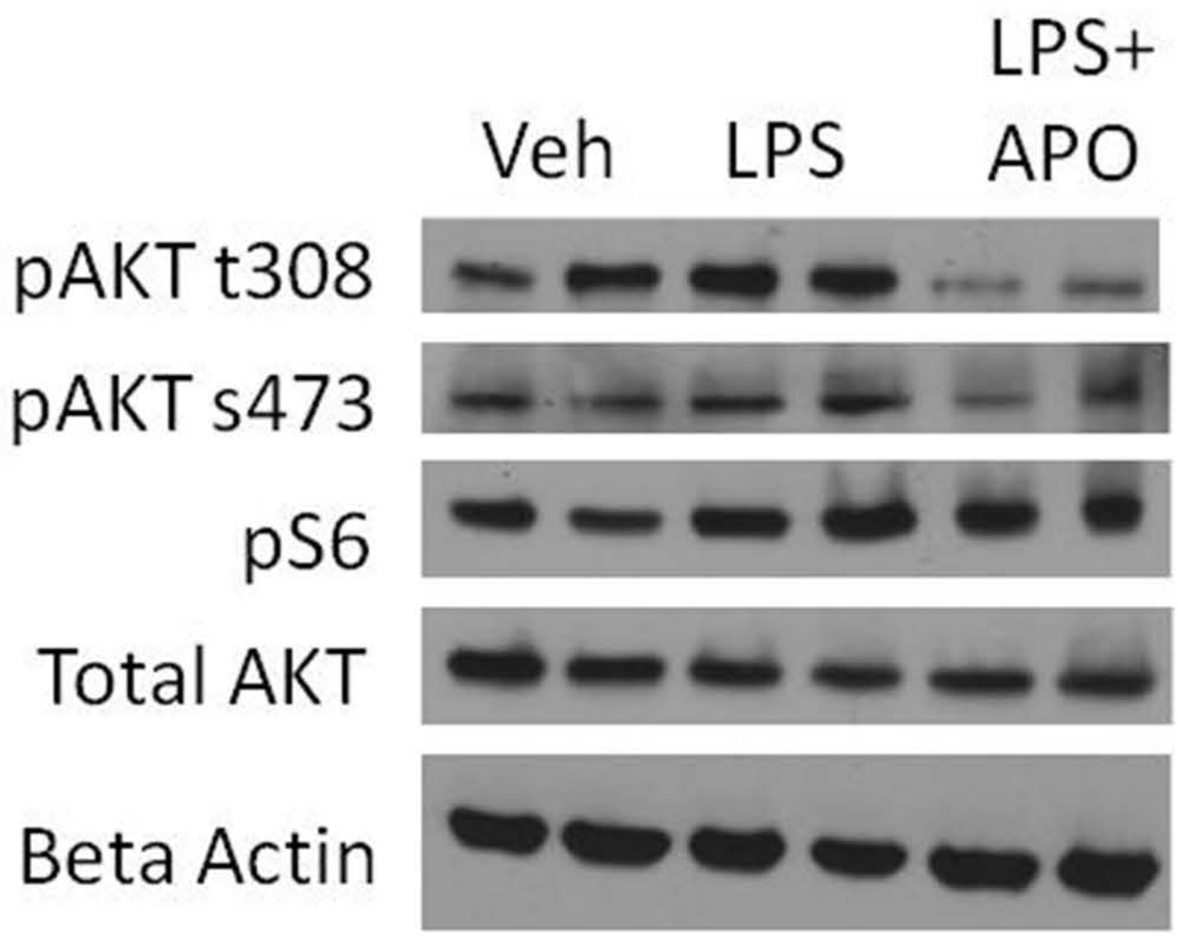
A


PTEN Heterozygous Brain Overgrowth
% Wild type littermates
140
120
100
80
60
40
20
0
LPS
LPS + APO
LPS
LPS + APO
Brain WW
% Body weight

B


Forebrain Microglia Number by Litter
% Total live cells
0.8
0.6
0.4
0.2
0
Veh
LPS
LPS + APO

C


PI3K/AKT Signaling Pathway Activation
Veh
LPS
LPS+ APO
pAKT t308
pAKT s473
pS6
Total AKT
Beta Actin

Supplementary Figure Legends

Figure S1. (A) The average number of pups born per litter is greater to LPS-treated dams compared to vehicle-treated dams. (B) The brain weights as a percentage of body weight of the male and female offspring from a single litter of MIR-exposed and control dams show no sex differences in response to treatment.. *P<0.05 All data expressed as Mean ± SEM.

Figure S2. (A) DAB immunostaining for IBA1+ microglia in the cortex and SVZ shows the diffuse localization of the cells throughout the forebrain. (B) Confocal images of Nestin and BrdU staining in the SVZ demonstrates the co-localization of the markers. (C) Semi-quantiative analysis of Nestin and BrdU co-labeling in the SVZ analyzed from confocal Z-stacks shows a greater overlap in MIR-exposed offspring. (D) Similar levels of MASH1 and BrdU co-labeling are seen in the SVZ of Veh and MIR exposed pups (E)) Stereologoical quantification of SVZ proliferation (BrdU) by gender shows no sex differences *P<0.05 All data expressed as Mean ± SEM.

Figure S3. Behavioral analysis of juvenile social approach to known and novel mice in the 3-chamber test shows a significantly less interest in novel targets (N=6 per group). *P<0.05 All data expressed as Mean ± SEM.

Figure S4. (A) Reduction of brain overgrowth in *PTEN* heterozygous mice with pre-natal NOX inhibition (N=1 het LPS and N=2 het LPS+APO) (B) The number of microglia as a percentage of total cells measured in the forebrain shows a significantly larger number in MIR-exposed compared to control pups at birth by litter (C) Western blot image of phospho-AKT, phospho-S6 and Beta-tubulin expression from acutely dissected SVZ tissue from MIR-exposed and control pups at birth shows increased pathway activation in the MIR-exposed pups despite the early (E9) treatment. *P<0.05 All data expressed as Mean ± SEM.

Supplemental Experimental Procedures

Cell culture. Standard neurosphere cultures and clonal density neurosphere assays were established for mouse SVZ and cortical cells according to the methods of Le Belle, et al., 2011.

Flow cytometry: Immunohistochemistry indicated that Iba1+ microglia were diffusely located throughout the forebrain with few cells located in or near the SVZ itself in both control and MIR-exposed pups (Fig. S2C). Therefore, whole forebrain dissociations and Iba1 antibody (AbCam) were used for quantification of microglia. SVZ micro-dissections were performed for the quantification of Ki67 (Leica) and nestin (BD Biosciences) co-labeling. The analysis of these populations was performed by FACS acquisition with a FACSDiVa cell sorter (BD Biosciences) using a purification-mode algorithm. Sort gates were set by side and forward scatter to eliminate dead and aggregated cells and by Alexa secondary fluorophores to define positive cells.

Western Blotting. The phospho-specific Akt antibody was purchased from Cell Signaling Technologies and the beta-tubulin control antibody was from Covance. Cells from acute SVZ dissections were lysed in buffer containing 0.1% triton X-100 in 50 mM Tris-HCl and 150 mM NaCl and Protease Inhibitor Cocktail (Sigma). Samples were prepared according to standard western blot protocol. Signal intensity was quantified using ImageJ software (NIH) and normalized to beta actin.

Measuring endogenous ROS levels. In vivo ROS levels were determined using the ROS-sensitive dye, hydroethidine (10 mg/kg; Invitrogen, Le Belle et al., 2011).

Behavioral Testing. The ultrasonic vocalization was performed on postnatal day 7 (P7). On P45-70 the 3-chamber social interaction and on P50-75 elevated plus maze tests (Banji, et al, 2011; Miura, et al., 2011) were performed on adult mice according to the methods of Crawley et al., 2012. The repetitive grooming test was performed in adult mice at P60 according to the methods of Peñagarikano, et al., 2011. Total path length was taken during the 3 chamber interaction test using Topscan (Cleversys Inc).

Supplemental References

Banji, D., Banji, O.J., Abbagoni, S., Hayath, M.S., Kambam, S., and Chiluka, V.L. (2011). Amelioration of behavioral aberrations and oxidative markers by green tea extract in valproate induced autism in animals. Brain Res. 1410, 141–151.

Miura, H., Ando, Y., Noda, Y., Isobe, K., and Ozaki, N. (2011). Long-lasting effects of inescapable-predator stress on brain tryptophan metabolism and the behavior of juvenile mice. Stress 14, 262–272.