

1 **Supplementary materials**

2 **Estrogen ameliorates microglial activation by inhibiting the Kir2.1**

3 **inward-rectifier K⁺ channel**

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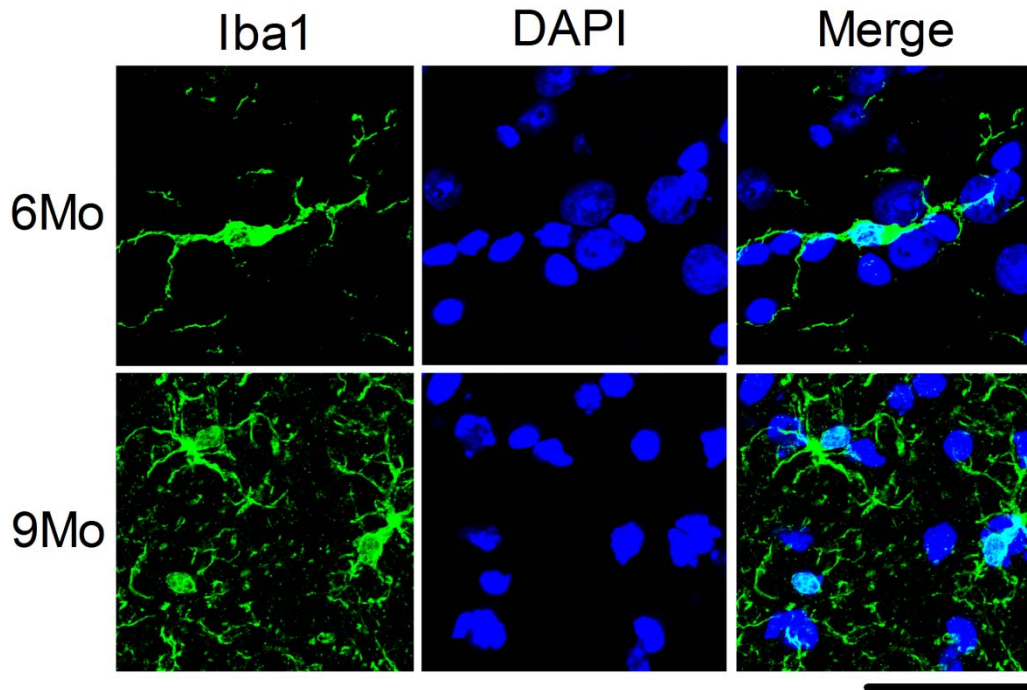
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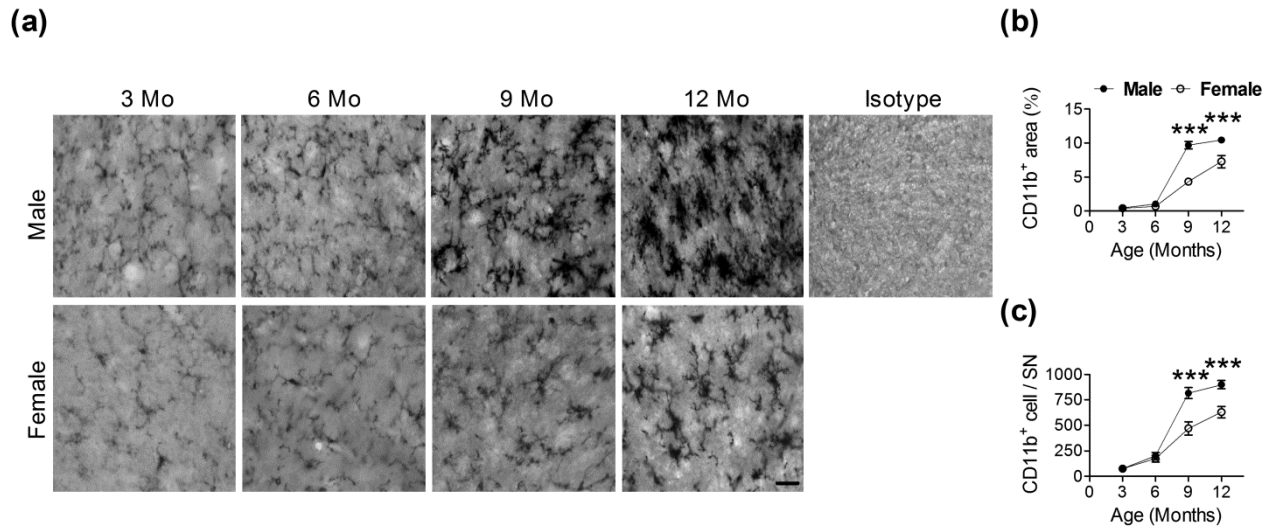


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Supplementary Figure S1. Confocal immunofluorescence microscopy images of Iba1⁺ cells in the SNs of 6- and 9-month-old male mice. Scale bar: 20 μ m. 6Mo: 6-month-old; 9Mo: 9-month-old.

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8 **Supplementary Figure S2. Temporal profiles of microglia in the SN of female and**

9 **male C57BL/6J mice. (a)** Representative immunostained micrographs show CD11b⁺

10 cells in the SN of 3-, 6-, 9-, and 12-month-old female and male mice. Scale bar: 10

11 μm . Quantitative results of CD11b⁺ cell areas **(b)** and the number of Iba1⁺ cells **(c)** in

12 the SN of female and male mice at different ages (n = 5). *** (p < 0.001): versus the

13 opposite sex.

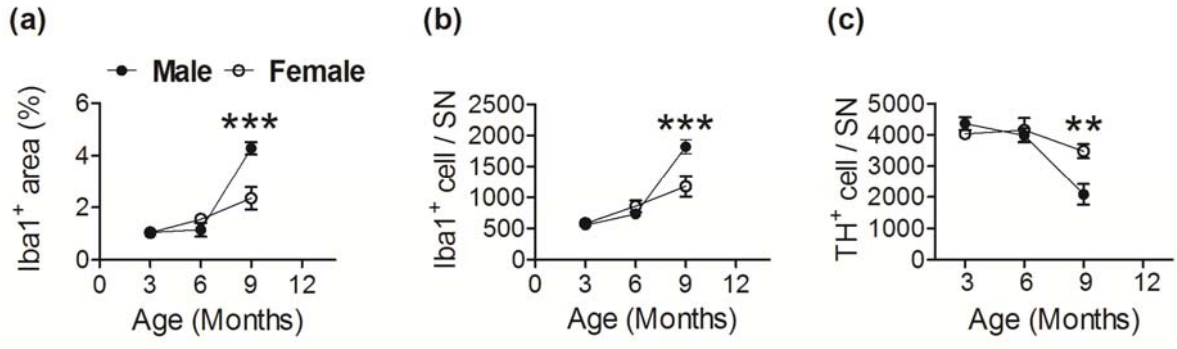
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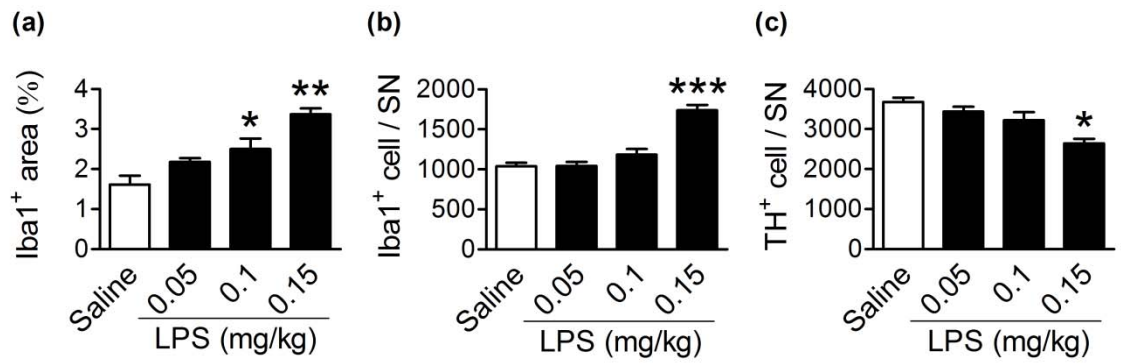


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8 **Supplementary Figure S3. Temporal profiles of microglia and DA neurons in the**
9 **SN of female and male BALB/c mice. (a,b,c)** Quantitative results of Iba1⁺ cell areas
10 **(a)** and the number of Iba1⁺ cells **(b)** and the number of TH⁺ cells **(c)** in the SN of
11 female and male mice at different ages (n = 4). ** (*p* < 0.01), *** (*p* < 0.001): versus
12 the opposite sex.

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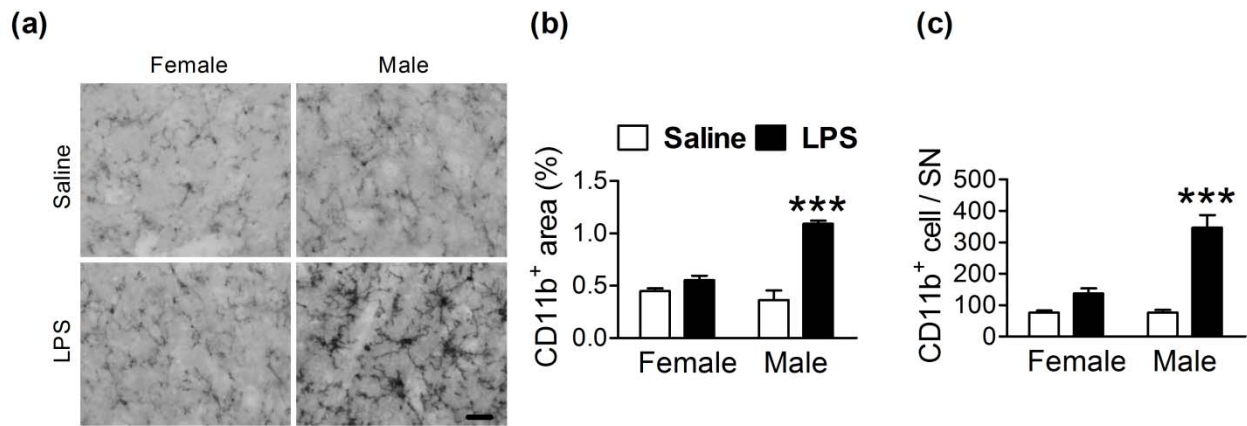
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Supplementary Figure S4. Dose effects of LPS on microglial activation and DA neuron loss in the SN of 3-month-old male mice. Dose effects of LPS on (a) Iba1⁺ cell areas, (b) the number of Iba1⁺ cells, and (c) the number of TH⁺ cells in the SN of 3-month-old male mice (n = 4). * (p < 0.05), ** (p < 0.01), *** (p < 0.001): versus Saline controls.

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8 **Supplementary Figure S5. Differential effects of LPS on microglial activation in**
9 **the SN of female and male mice.** (a) Representative immunostained micrographs
10 show CD11b⁺ cells in the SN of female and male mice 1 day after an LPS (0.15
11 mg/kg of body weight, i.p.) injection. Scale bar: 10 μ m. Quantitative results of
12 CD11b⁺ cell areas (b) and the number of CD11b⁺ cells (c) in the SN of female and
13 male mice 1 day after an LPS injection (n = 5-6). *** ($p < 0.001$): versus respective
14 Saline group.

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1 **Supplementary Table S1. Body weight of C57BL/6J mice.**

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Age (months)		Sample size	Mean \pm SEM (g)	coefficient of variation (%)
3	male	8	21.7 \pm 0.7	9.2
	female	10	21.4 \pm 0.3	4.8
6	male	8	26.2 \pm 0.6	6.2
	female	7	25.5 \pm 0.8	8.4
9	male	8	29.7 \pm 0.6	5.6
	female	5	28.2 \pm 0.6	4.9
12	male	4	33.3 \pm 1.1	6.6
	female	7	29.6 \pm 0.8	7.5

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1 **Supplementary Table S2. The health monitoring annual report of C57BL/6J**
 2 **mice.**

Laboratory Animal Center of National Cheng Kung University
 Health monitoring report

Location : Tainan, Taiwan

Report date : 25 Jan,2016

Area : 12F Clean

ROOM : CH32-H

Specics : Mouse

	Current POS/TSTD	12Months POS/TSTD	Methods
Mouse Hepatitis virus	0/1	0/5	ELISA
Pneumonia Virus of Mice	0/1	0/5	ELISA
TMEV	0/1	0/5	ELISA
Mouse adenovirus (MAd)	0/1	0/5	ELISA
Mouse parvovirus (MPV)	0/1	0/5	ELISA
Mycoplasma pulmonis	0/1	0/5	ELISA
Murine norovirus	0/1	0/5	ELISA
Minute virus of mice (MVM)	0/1	0/5	ELISA
Hantaan virus	0/1	0/2	ELISA
Sendai virus	0/1	0/3	ELISA
Ectromelia virus (Mouse Pox)	0/1	0/3	ELISA
Reovirus 3 (Reo 3)	0/1	0/3	ELISA
LCMV	0/1	0/2	ELISA
<i>Bordetella bronchiseptica</i>	0/1	0/3	Culture
<i>Corynebacterium kutscheri</i>	0/1	0/3	Culture
<i>Salmonella</i> spp.	0/1	0/3	Culture
<i>Pseudomonas aeruginosa</i>	0/1	0/3	Culture
<i>Helicobacter</i> spp.	0/1	0/3	PCR
<i>Pneumocystis murina</i>	0/1	0/3	PCR
Endoparasites- Helminths			
<i>Aspicularis tetraptera</i>	0/1	0/3	Direct exam. and flotation test
<i>Hymenolpis diminuta</i>	0/1	0/3	Direct exam. and flotation test
<i>Rodentolepis nana</i>	0/1	0/3	Direct exam. and flotation test
<i>Syphacia</i> spp.	0/1	0/3	Direct exam. and flotation test
Ectoparasites			
Flea	0/1	0/3	Clinical exam and necropsy
Louse	0/1	0/3	Clinical exam and necropsy
Mite	0/1	0/3	Clinical exam and necropsy

