

## Supplementary Materials

# Sleep Deprivation Triggers Mitochondrial DNA Release in Microglia to Induce Neural Inflammation: Preventative Effect of Hydroxytyrosol Butyrate

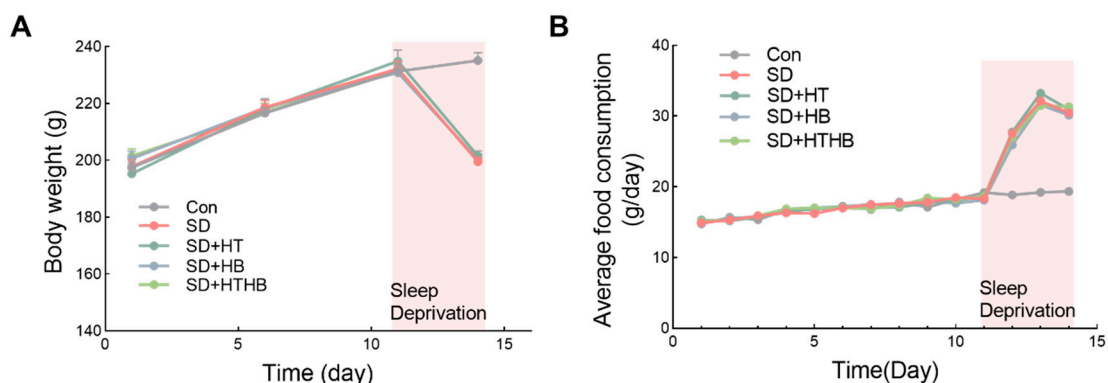
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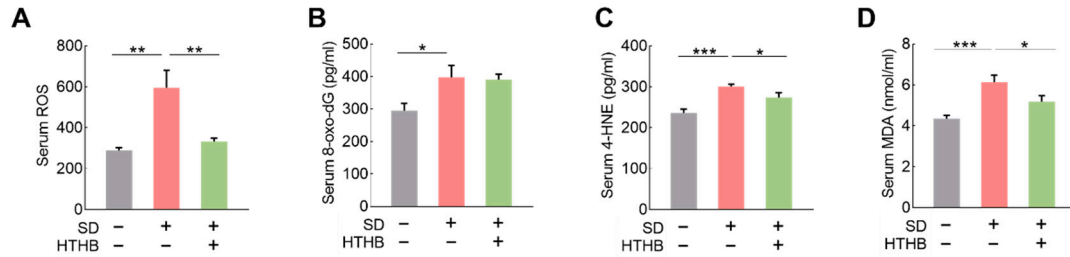
This file includes:

Figures S1–S7

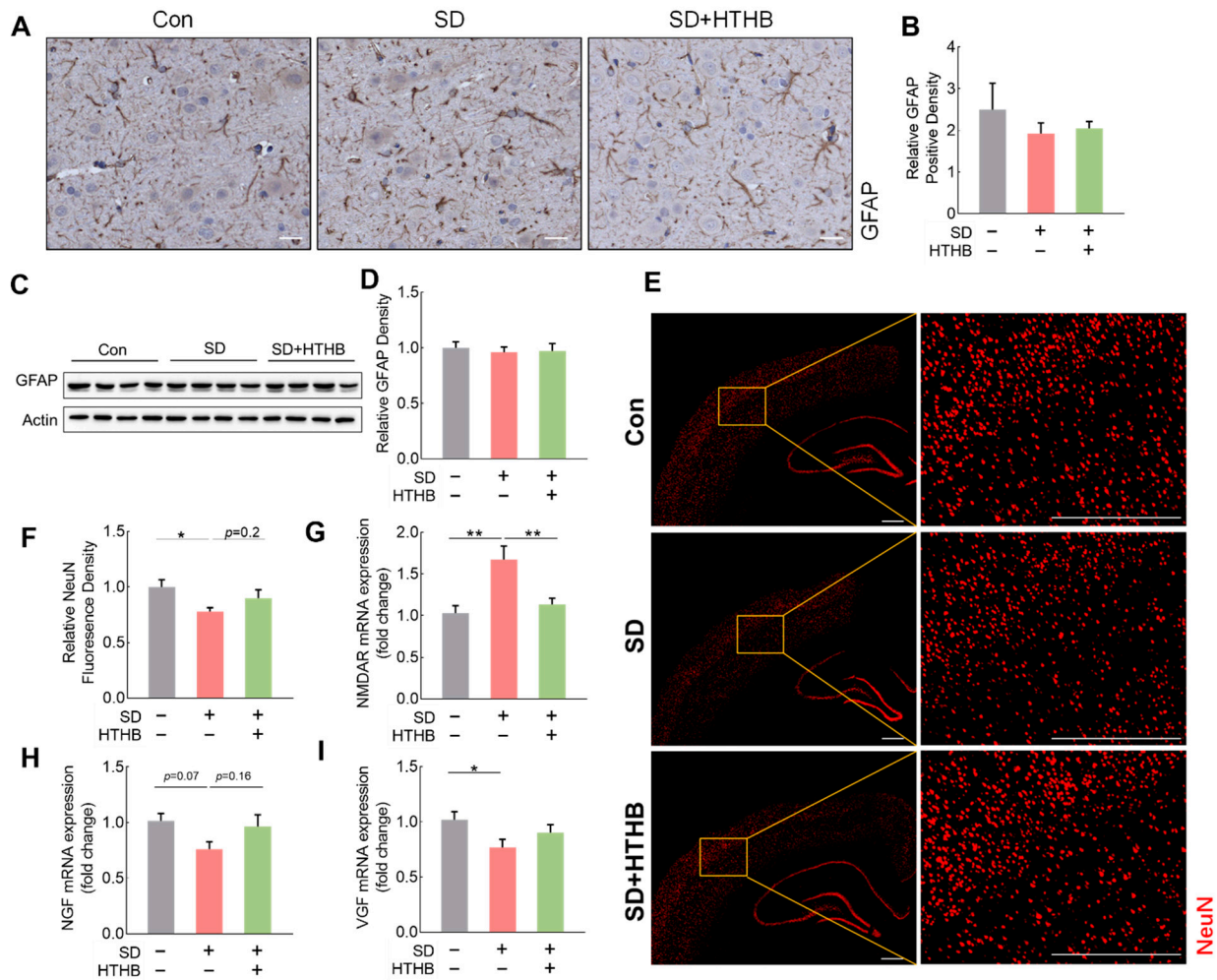
Tables S1



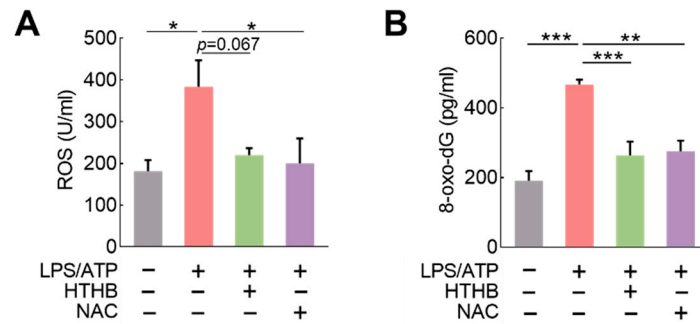
**Figure S1.** Changes in body weight and food consumption before and after the sleep deprivation. (A) Body weight, and (B) food consumption, in control (Con), sleep deprivation (SD), SD with hydroxytyrosol (HT), SD with b hydroxybutyric acid (HB), and SD with hydroxytyrosol butyrate (HTHB).



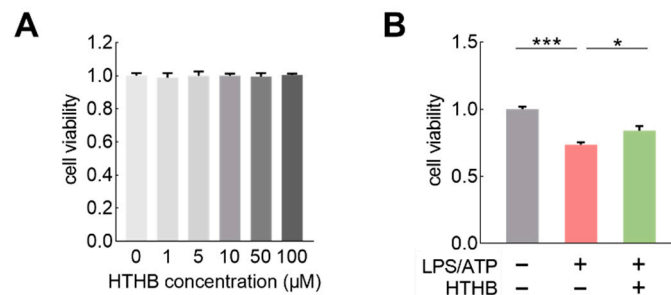
**Figure S2.** Sleep deprivation increases the oxidative stress in serum, which is partially attenuated by HTHB. (A, B) Serum levels of reactive oxygen species (ROS) (A) and 8-oxo-dG (B). (C, D) The levels of 4-hydroxynonenal (4-HNE) (C) and malondialdehyde (MDA) (D) in the serum of rats. Values are mean  $\pm$  SEM,  $n = 4$ , \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . The results were analyzed using one-way ANOVA.



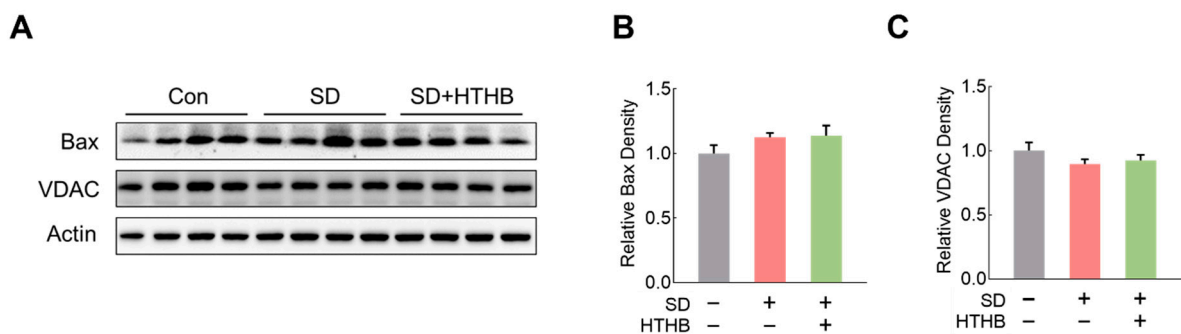
**Figure S3.** Sleep deprivation does not affect astrocytes but induces neuronal loss and affects neuronal function, which are alleviated by HTHB in the rat brain. (A) Immunohistochemistry of glial fibrillary acidic protein (GFAP), a marker of activated astrocyte, in control, after sleep deprivation (SD), and SD+HTHB in the cortex of rat brain. Scale = 25  $\mu$ m,  $n = 4$ . (B) The relative GFAP-positive densities. (C) The typical example of Western blots of GFAP. (D) The relative GFAP density in control, SD, SD+HTHB in the rat brain,  $n = 8$ . (E) Immunofluorescent staining of NeuN (red) in rat brain. Scale = 500  $\mu$ m,  $n = 4$ . (F) Relative immunofluorescent intensity of NeuN. (G-I) mRNA levels of N-methyl-D-aspartate receptor (NMDAR) (G), nerve growth factor (NGF) (H), and VGF nerve growth factor inducible (VGF) (I) in the rat cortex,  $n = 8$ . Values are mean  $\pm$  SEM,  $n \geq 3$ , \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . The results were analyzed using one-way ANOVA.



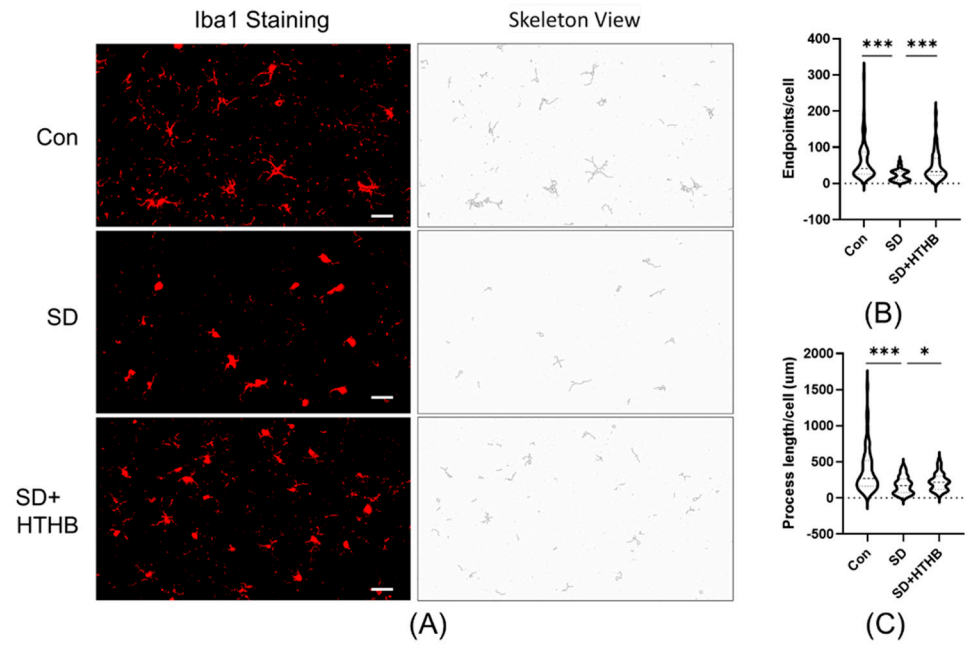
**Figure S4.** Effects of HTHB and N-acetyl cysteine (NAC) on LPS/ATP-induced ROS and 8-oxo-dG in N9 cells. **(A,B)** LPS/ATP treatment significantly increase the level of ROS (A) and 8-oxo-dG Levels (B), which are attenuated by both HTHB (10  $\mu$ M) and NAC (0.5 mM). Values are mean  $\pm$  SEM, n = 4, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001. The results were analyzed using one-way ANOVA.



**Figure S5.** The effects of HTHB on N9 cell viability. **(A)** Treatment with HTHB at different concentrations did not affect the cell viability of N9 microglial cells after 48 h. **(B)** Treatment with HTHB (10  $\mu$ M) for 48 h attenuated the LPS/ATP-induced cell death of N9 microglial cells. Values are mean  $\pm$  SEM, n = 8, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001. The results were analyzed using two-way ANOVA or one-way ANOVA.



**Figure S6.** Expressions of Bax microchannel and voltage dependent anion channel (VDAC) were not affected by either sleep deprivation nor HTHB. **(A)** Typical example of Western blot of Bax and VDAC in the rat brain. **(B, C)** Relative expression densities of Bax (B) and VDAC (C). Values are mean  $\pm$  SEM, n = 8, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001. The results were analyzed using one-way ANOVA.



**Figure S7.** SD affects microglia morphology, which are ameliorated by HTHB. **(A)** Immunofluorescence staining of Iba1 and relative skeleton view. Scale bar = 20  $\mu$ m. **(B)** Microglia endpoints per cell. **(C)** Process length per cell. Values are mean  $\pm$  SEM, n = 30 (from 3 rat brain slices), \*p < 0.05, \*\*\*p < 0.001. The results were analyzed using one-way ANOVA.

**Table S1.** Primer list.

| Species | Gene           | Primer (5'-3')   |
|---------|----------------|--|
| Rat     | <i>β-actin</i> | Forward: AGATCAAGATCATTGCTCCTCCT<br>Reverse: ACGCAGCTCAGTAACAGTCC      |
| Rat     | <i>NGF</i>     | Forward: CATCGCTCTCCTTCACAGAGTT<br>Reverse: ATTACGCTATGCACCTCAGAGT     |
| Rat     | <i>NMDAR</i>   | Forward: GAAAACCTCGACCAACTGTCC<br>Reverse: GTCGTCCTCGCTTGCAGA          |
| Rat     | <i>VGF</i>     | Forward: CCCAGAACGAGGATTGCGAG<br>Reverse: CTGCCAGAGACTCCGAAGAG         |
| Rat     | <i>TNF</i>     | Forward: ATGGGCTCCCTCTCATCAGT<br>Reverse: GCTTGGTGGTTTGCTACGAC         |
| Rat     | <i>IL6</i>     | Forward: CTCTCCGCAAGAGACTTCCAG<br>Reverse: TGTGGGTGGTATCCTCTGTGA       |
| Rat     | <i>IL1β</i>    | Forward: CAGCTTTCGACAGTGAGGAGA<br>Reverse: TTGTCGAGATGCTGCTGTGA        |
| Rat     | <i>18s</i>     | Forward: CGAACGTCTGCCCTATCAACTT<br>Reverse: CTGGATGTGGTAGCCGTTTCT      |
| Rat     | <i>D-loop</i>  | Forward: AATCTACCATCCTCCGTG<br>Reverse: GACTAATGATTCTTCACCGT           |
| Mouse   | <i>β-actin</i> | Forward: GTTGGTTGGAGCAAACATC<br>Reverse: CTTATTTTCATGGATACTTGGAATG     |
| Mouse   | <i>loop1</i>   | Forward: AATCTACCATCCTCCGTGAAACC<br>Reverse: TCAGTTTAGCTACCCCCAAGTTTAA |
| Mouse   | <i>loop2</i>   | Forward: CCCTTCCCCATTGGTCT<br>Reverse: TGGTTTCACGGAGGATGG              |
| Mouse   | <i>loop3</i>   | Forward: TCCTCCGTGAAACCAACAA<br>Reverse: AGCGAGAAGAGGGGCATT            |
| Mouse   | <i>loop4</i>   | Forward: AACGGATCCACAGCCGTA<br>Reverse: AGTCCTTCGGGCCATGATT            |
| Mouse   | <i>IL6</i>     | Forward: TAGTCCTTCCTACCCCAATTTCC<br>Reverse: TTGGTCCTTAGCCACTCCTTC     |
| Mouse   | <i>TNFα</i>    | Forward: CCCTCACACTCAGATCATCTTCT<br>Reverse: GCTACGACGTGGGCTACAG       |