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Supplementary data to:

DEOXYNIVALENOL ENHANCES IL-1ß EXPRESSION IN BV2 MICROGLIAL CELLS THROUGH ACTIVATION OF THE NF-KB PATHWAY AND THE ASC/NLRP3 INFLAMMASOME

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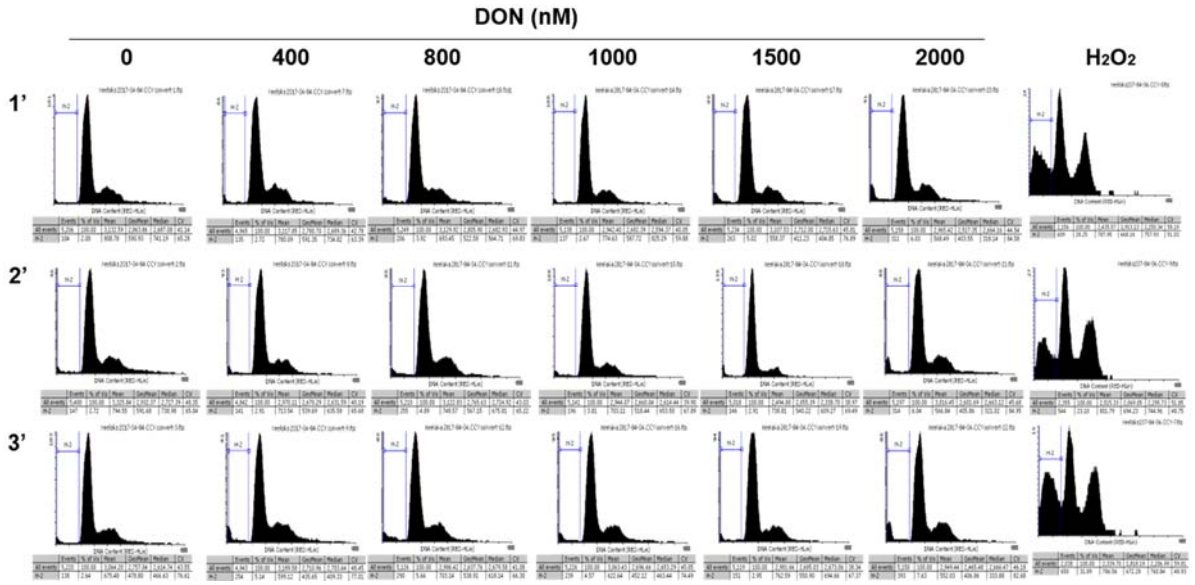

Supplementary Figure 1: These are supplementary data for Figure 1C. BV2 microglial cells were seeded at a density of 1 × 10^5 cells/mL and incubated with various concentrations of DON for 24 h. The percentages of sub-G_1 DNA content were analyzed by flow cytometry and the images are representative analyses.

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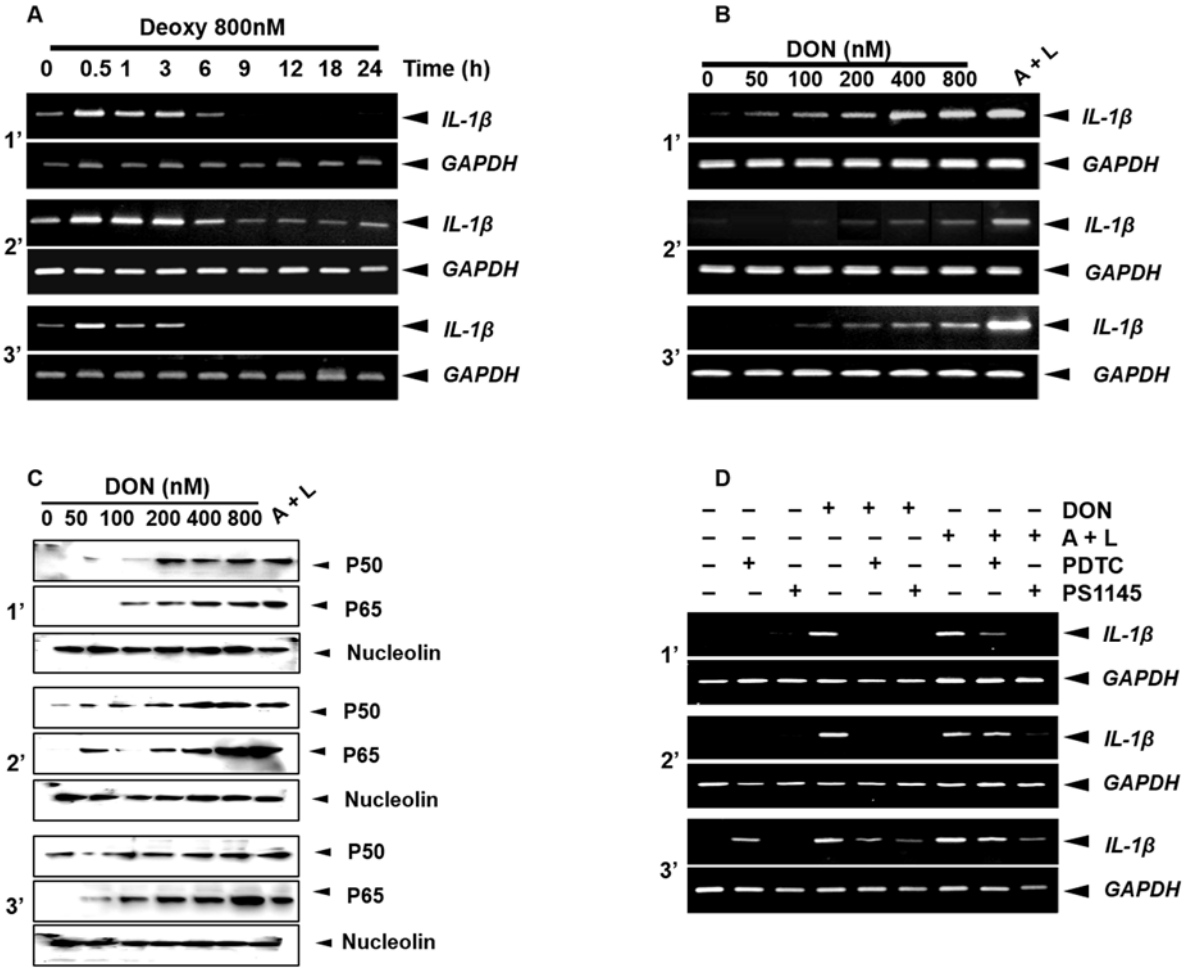

Supplementary Figure 2: These are all triplicate experiments for Figure 2. (**A**) BV2 microglial cells (1 × 10^5 cells/mL) were treated with 800 nM DON and harvested at the indicated time points. Total cellular RNA was subjected to RT-PCR and the PCR products were separated on a 2 % agarose gel. (**B**) The cells were treated with the indicated concentrations of DON or 1 mM ATP and 100 ng/mL LPS for 1 h. The extracted mRNA was then subjected to RT-PCR and the PCR products were separated on a 2 % agarose gel. (**C**) In a parallel experiment, the cells were treated with the indicated concentrations of DON or 1 mM ATP and 100 ng/mL LPS for 30 min, after which the nuclear compartment was purified and western blotting for p50 and p65 was performed; nucleolin was used as a control nuclear protein. (**D**) For the functional analysis of NF-κB, BV2 microglial cells were pre-incubated with 10 μM PDTC and 10 μM PS1145 for 1 h and then treated with 800 nM DON or 1 mM ATP and 100 ng/mL LPS. Total cellular RNA was subjected to RT-PCR analysis for *IL-1β* expression.

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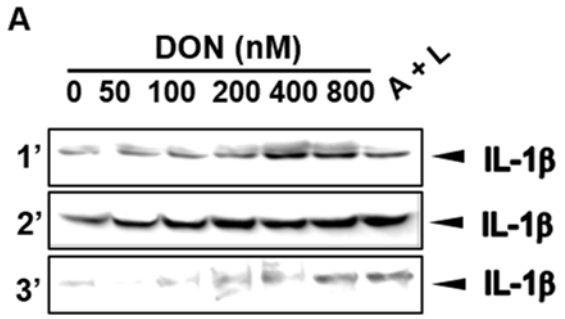

Supplementary Figure 3: These are all triplicate experiments for Figure 3A. BV2 microglial cells (1 × 10^5 cells/mL) were treated with the indicated concentrations of DON or 1 mM ATP and 100 ng/mL LPS for 24 h and the culture media was collected. Western blotting analysis was performed to measure active IL-1β secretion.

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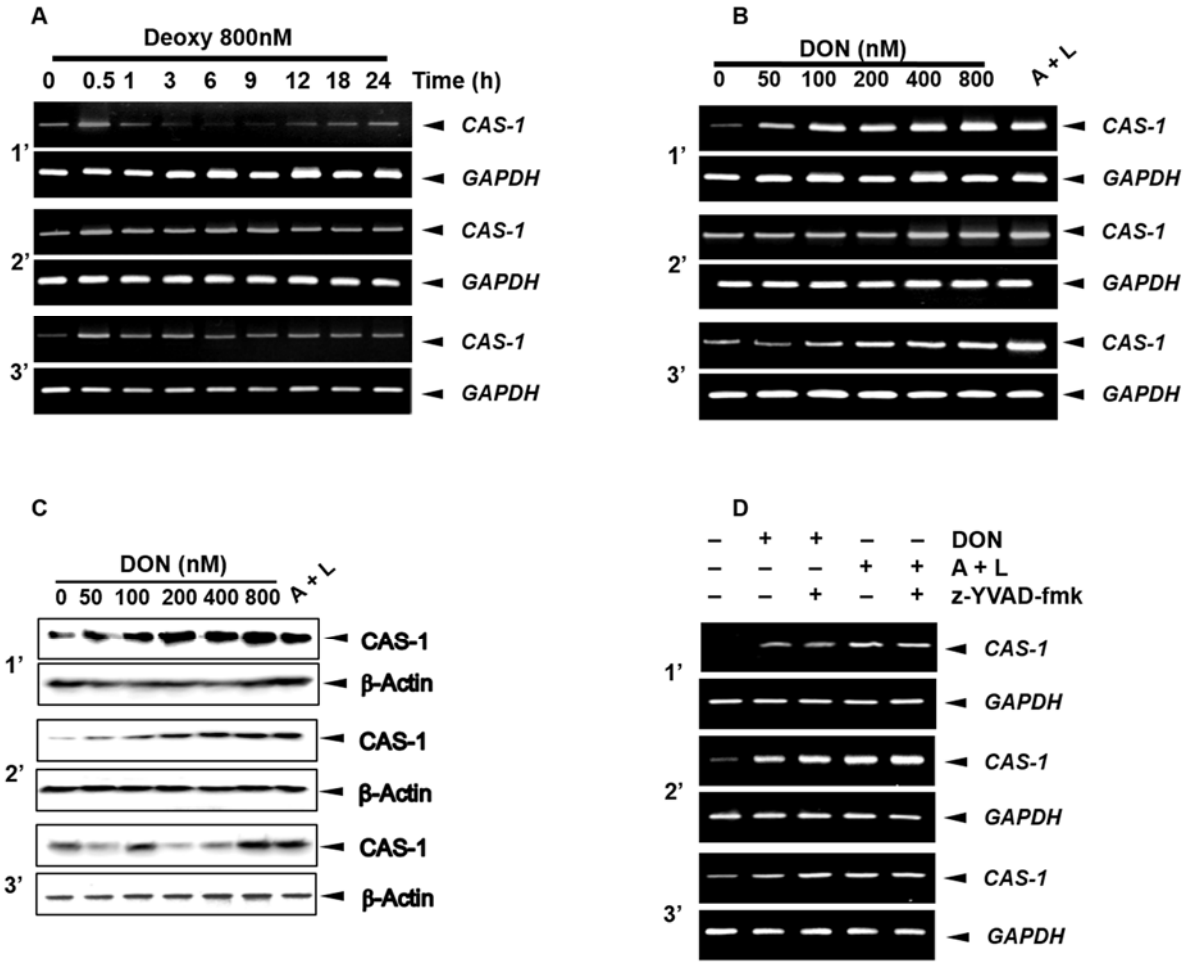

Supplementary Figure 4: These are all triplicate experiments for Figure 4. (**A**) BV2 microglial cells were seeded at a density of 1 × 10^5 cells/mL, treated with 800 nM DON, and harvested at the indicated time points. RT-PCR analysis was conducted to assess the time course for *caspase-1* expression. (**B**) The cells were treated with the indicated concentrations of DON or 1 mM ATP and 100 ng/mL LPS. The effect of DON on *caspase-1* expression in BV2 microglial cells was assessed by RT-PCR. (**C**) In a parallel experiment, western blotting for caspase-1 was performed. (**D**) Cells were pretreated with z-YVAD-fmk (10 μM) 2 h before treatment with DON or 1 mM ATP and 100 ng/mL LPS, and *caspase-1* expression was determined by RT-PCR.

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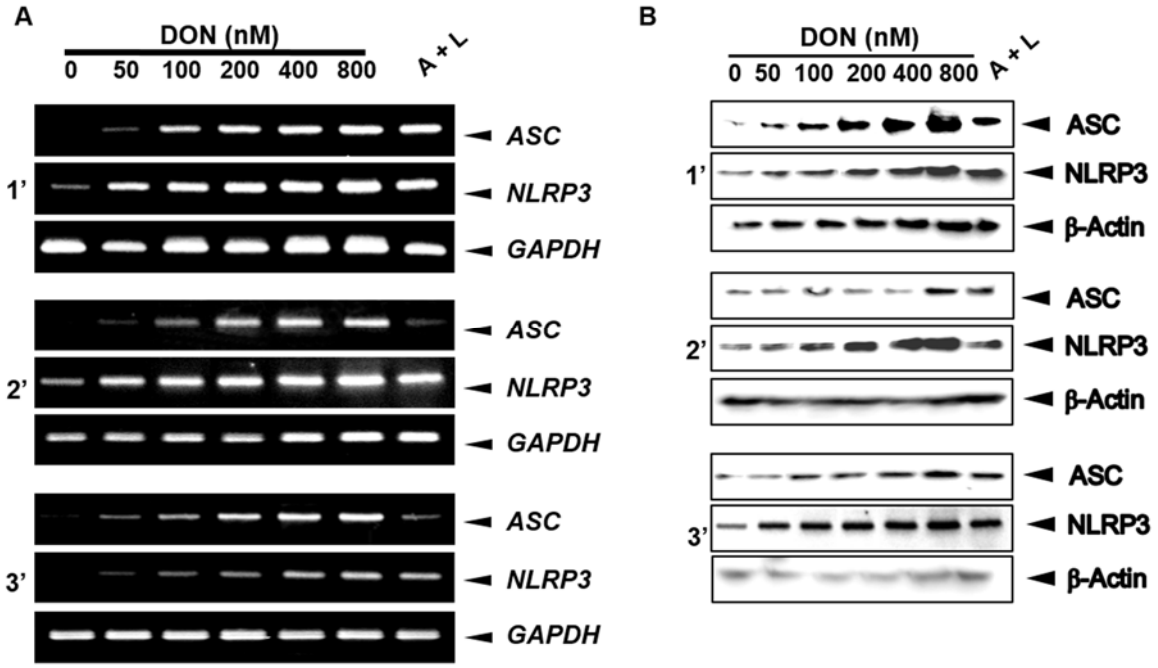

Supplementary Figure 5: These are all triplicate experiments for Figure 5. BV2 microglial cells were seeded at a density of 1 × 10^5 cells/mL and treated with the indicated concentrations of DON or 1 mM ATP and 100 ng/mL LPS. (**A**) At 1 h after the administration of DON, the effect on *ASC* and *NLRP3* expression was assessed by RT-PCR. (**B**) The cytosolic fraction of BV2 microglial cell lysate was used to assess ASC and NLRP3 protein expression after 24 h.

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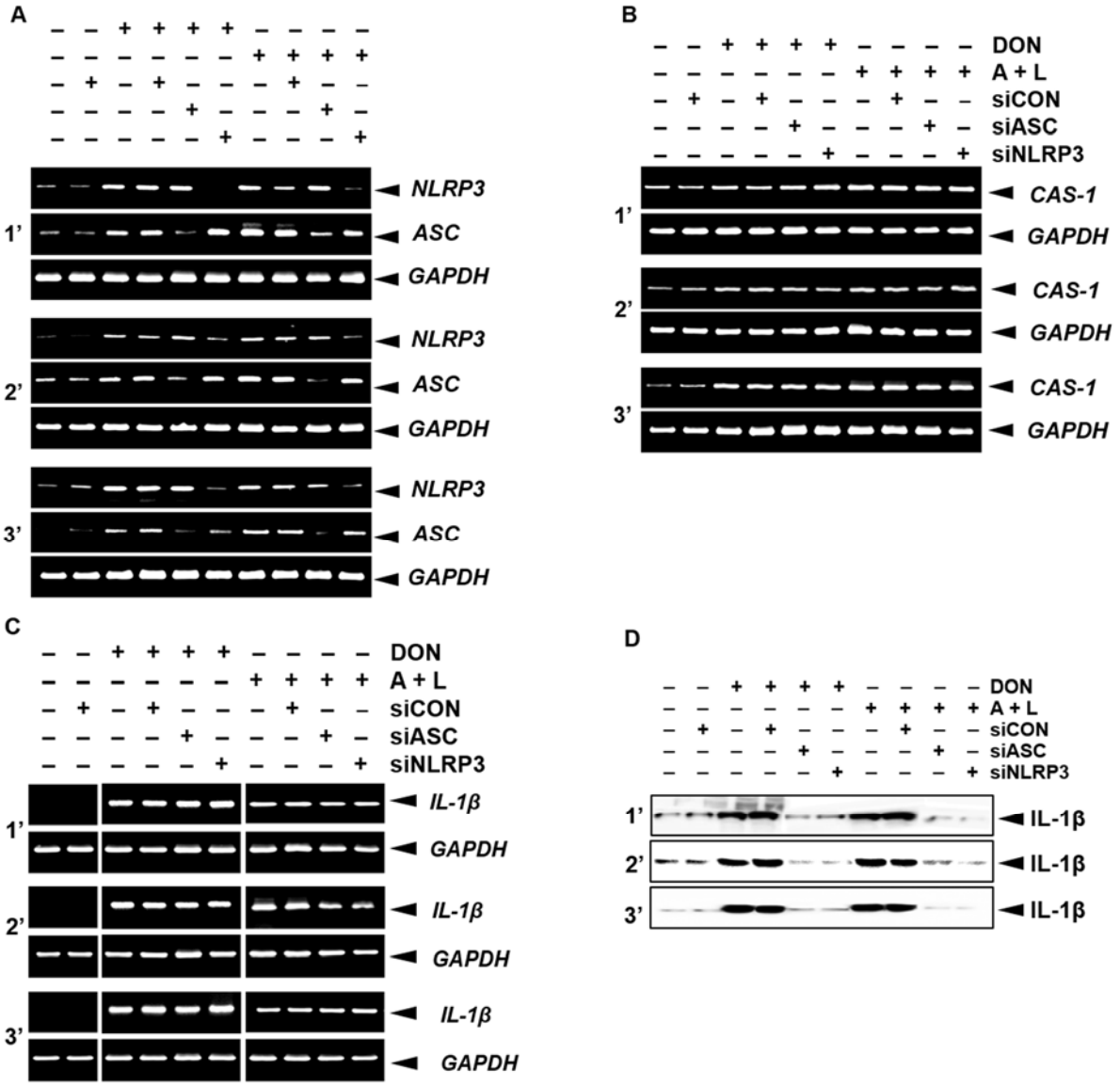

Supplementary Figure 6: These are all triplicate experiments for Figure 6. BV2 microglial cells were seeded at the density of 1 × 10^5 cells/mL overnight and then transfected with siASC and siNLRP3 for 48 h. The cells were treated with DON (800 nM), or 1 mM ATP and 100 ng/mL LPS. (A) The effect of DON on ASC and NLRP3 expression was assessed by RT-PCR 1 h after treatment with DON, or ATP and LPS. (**B** and **C**) In a parallel experiment, caspase-1 (**B**) and pro-IL-1β (**C**) expression was detected by RT-PCR at samples taken 1 h after treatment. (**D**) The cytosolic expression of IL-1β was detected by western blotting at 24 h. CAS-1; caspase-1. A + L; treatment with 1 mM ATP and 100 ng/mL LPS.