

**Supplementary Materials for**  
**Anti-Inflammatory Effects of Idebenone Attenuate Systemic Inflammatory Diseases by**  
**Suppression of NF- $\kappa$ B Activation.**

Yumin Choi<sup>1,2§</sup>, Young-Lai Cho<sup>1,2§</sup>, Sujeong Park<sup>1,2</sup>, Minkyung Park<sup>1,2</sup>, MinJeong Kim<sup>1,2</sup>,  
Young Jun Park<sup>1,2</sup>, In-Ah Lee<sup>3</sup>, Su Wol Chung<sup>4</sup>, Heedoo Lee<sup>5</sup>, Seon-Jin Lee<sup>1,2</sup>

<sup>1</sup> Environmental Disease Research Center, Korea Research Institute of Bioscience and  
Biotechnology (KRIBB), Daejeon, 34141, Republic of Korea

<sup>2</sup> Department of Functional Genomics, University of Science and Technology (UST), Daejeon,  
34113, Korea

<sup>3</sup> Department of Chemistry, Kunsan National University, Gunsan 54150, South Korea

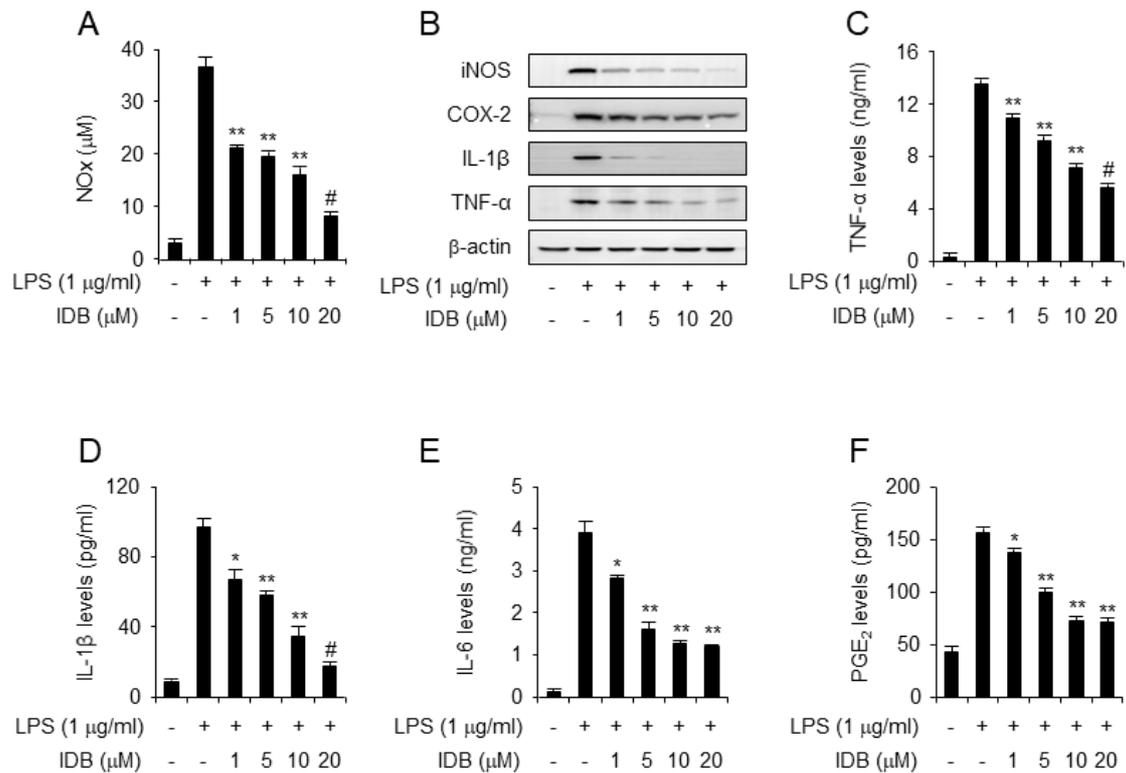
<sup>4</sup> Department of Biological Sciences, College of Natural Sciences, University of Ulsan, 93  
Daehak-ro, Nam-gu, Ulsan 44610, South Korea

<sup>5</sup> Department of Biology and Chemistry, Changwon National University, Changwon 51140,  
South Korea.

§These authors contributed equally to this work

**Corresponding Author:** Seon-Jin Lee, Ph.D., Environmental Disease Research Center,  
Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, South Korea.

Tel: +82-42-879-8293; E-mail: [sjlee@kribb.re.kr](mailto:sjlee@kribb.re.kr)



**Supplementary Figure 1.** Idebenone inhibits LPS-stimulated expression of proinflammatory mediators in J774A.1 cells. Idebenone inhibits LPS-induced production of NO metabolites, TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and PGE<sub>2</sub> in J774A.1 cells. (A) NO metabolite secretion was assessed using Griess reagent. (B) Western blotting analysis was used to examine protein expression of inflammatory enzymes (iNOS, COX-2) and pro-inflammatory cytokines (IL-1 $\beta$ , TNF- $\alpha$ ) in LPS-induced J774A.1 cells in the presence or absence of various doses of idebenone (1, 5, 10, 20  $\mu$ M), with  $\beta$ -actin as the loading control. Cells were pretreated with idebenone (1, 5, 10, 20  $\mu$ M) for 2 h before LPS treatment (1  $\mu$ g/mL). Cells were harvested after 18 h. The secretion of (C) TNF- $\alpha$ , (D) IL-1 $\beta$ , (E) IL-6, and (F) PGE<sub>2</sub> by LPS-stimulated J774A.1 cells treated with or without idebenone was assessed using ELISA. Graphs indicate the mean of three independent experiments. Statistical analysis was performed using paired two-tailed Student's *t*-test. \**p* < 0.05, #*p* < 0.001.