Supplementary methods

Quantification of nitric oxide (NO) production. NO levels in culture supernatants were determined using the Griess reaction in a 24-well plate format. Briefly, MH-S cells were pretreated with GBD (10-20 μM) or 0.1% DMSO for 30 min, followed by stimulation with LTA (10 μg/ml) or left unstimulated for 24 h. After incubation, supernatants were collected and mixed 1:1 with freshly prepared Griess reagent (1% sulfanilamide and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride in 2.5% phosphoric acid). The mixture was incubated at room temperature to allow color development, and absorbance was measured at 540 nm using a microplate spectrophotometer (MRX; Dynex Technologies Inc.). Sodium nitrite was used as a standard to generate a calibration curve for quantification.

Malondialdehyde (MDA) assay. Lipid peroxidation in MH-S cells was assessed by quantifying MDA levels using a TBARS Assay Kit (cat no. 10009055; Cayman Chemical Company) according to the manufacturer's instructions.

Figure S1. Effect of GBD on LTA-induced proliferation of MH-S cells. Cells were pretreated with GBD for 30 min, followed by LTA stimulation (10 μg/ml) for 24 h. Cell proliferation was assessed by the MTT assay.

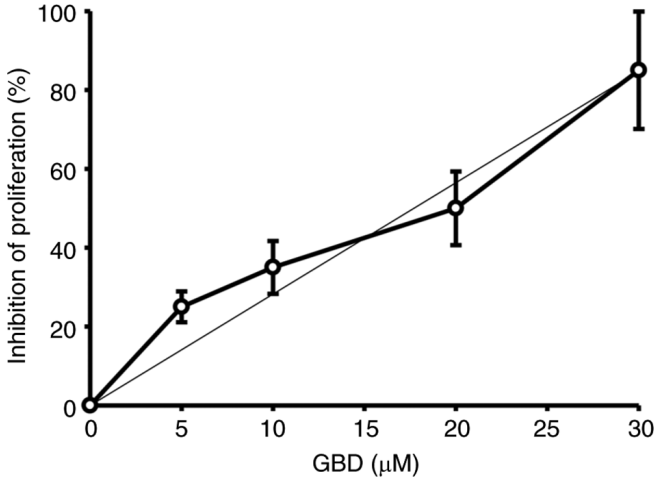

Figure S2. Knockdown efficiency of Nrf2 siRNA in MH-S cells. Cells were transfected with Nrf2-specific siRNA (siNrf2) of negative control siRNA (si-Ctrl) for 24 h. Nrf2 mRNA expression was measured by RT-qPCR. Data are presented as mean ± SD (n=3). ***P<0.001 vs. si-Ctrl.

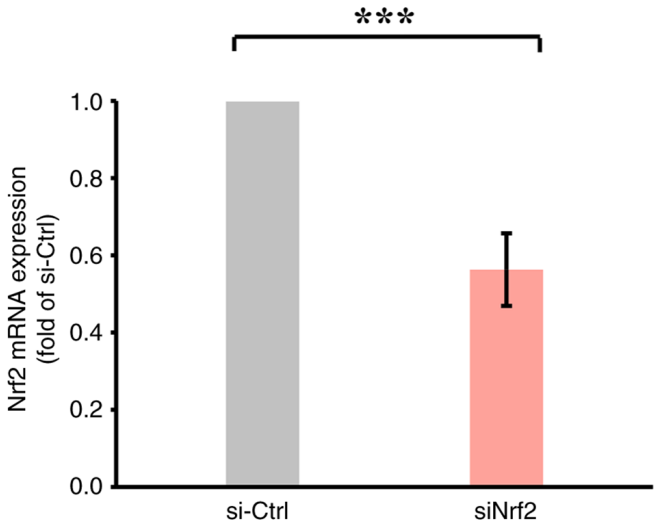

Figure S3. Inflammatory mediators and oxidative stress markers in MH-S cells. Cells were pretreated with GBD (20 μM) or 0.1% DMSO for 30 min, followed by LTA (10 μg/ml) stimulation. (A-D) mRNA expression of IL-1β, TNF-α, IL-6, and SOD1 was measured at 6 h after LTA stimulation. (E) Malondialdehyde (MDA) levels were measured in cell lysates after 24 h of LTA stimulation. (F) Nitric oxide (NO) production was determined at 24 h using the Griess assay. Data are presented as mean ± SD (n=3). ***P<0.001 vs. DMSO; $^{\#\#}$P<0.01, and $^{\#\#\#}$P<0.001 vs. DMSO+LTA.