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Supplemental Data

N,N-Dimethylacetamide Significantly Attenuates LPS- and TNFα-Induced Proinflammatory Responses Via Inhibition of the Nuclear Factor Kappa B Pathway

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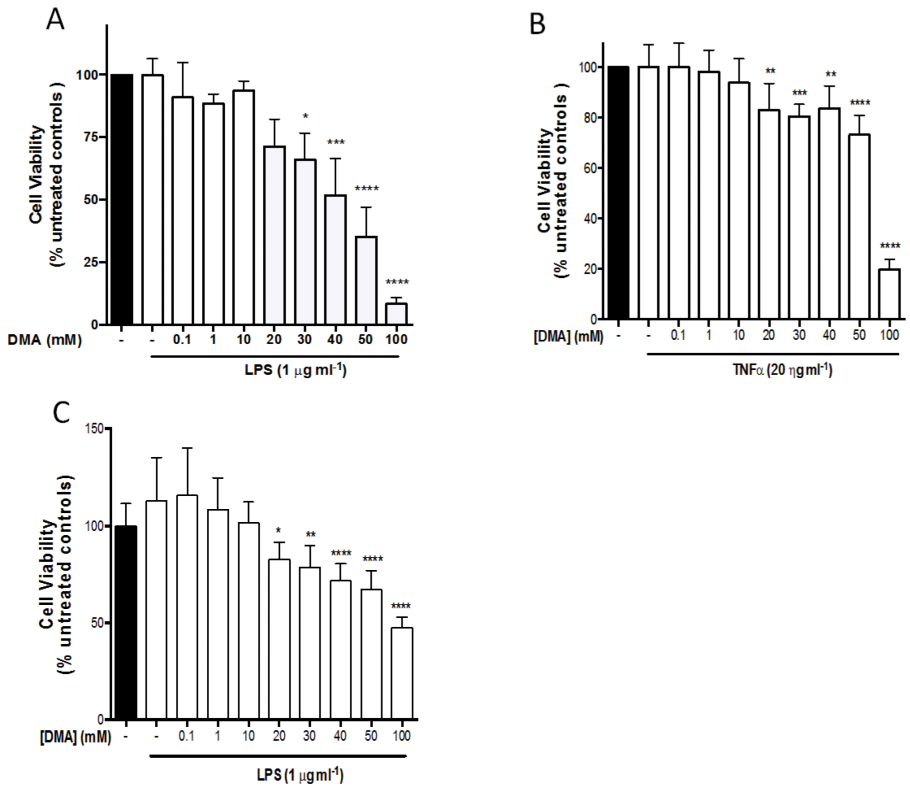
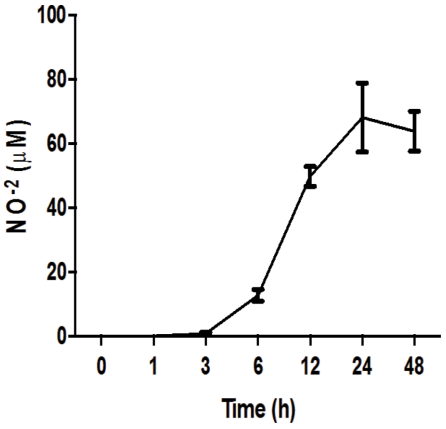
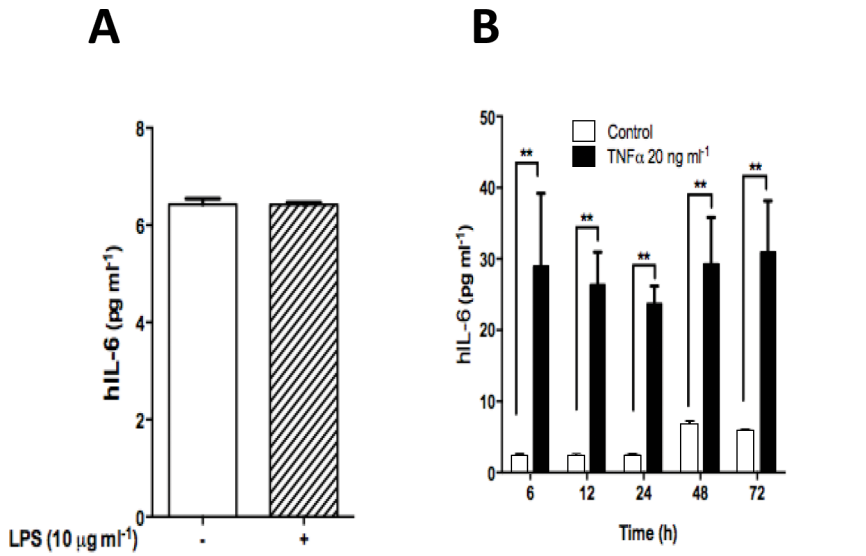


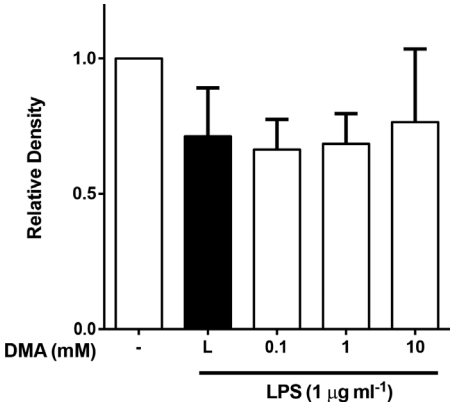
Supplementary Figure S1. MTT cell viability assays. (A) RAW 264.7 cells, (B) JEG-3 cells (C) HEK 293/TLR4 cells. Increasing concentrations of DMA were used as indicated to determine the highest noncytotoxic concentration of DMA in the presence of LPS (RAW 264.7 cells and HEK 293/TLR4 cells) or TNFα (JEG-3 cells). Data shown are means ± SEM of at least three independent experiments done in quadruplicate wells. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$.



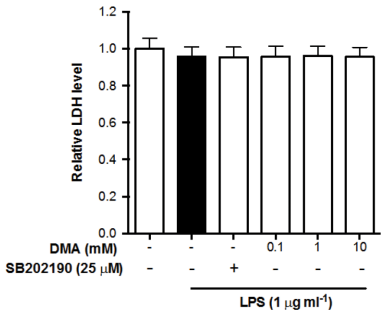
Supplementary Figure S2. NO^{-2} secretion time course. NO^{-2} secretion was determined by Griess assay in RAW 264.7 cells after incubation with 1 μg/mL LPS at varying time points to optimize the DMA inhibition assay.



Supplementary Figure S3. JEG-3 cells secrete IL-6 in response to TNFα but not LPS. (A) ELISA was performed to determine IL-6 levels in JEG-3 cell supernatants after incubation in the absence or presence of LPS. (B) ELISA was performed to determine IL-6 levels in JEG-3 supernatants in the absence or presence of TNFα at varying time points, as shown. Data shown are means ± SEM of at least three independent experiments done in duplicate. $^{**}P < 0.01$.



Supplementary Figure S5. DMA decreases LPS-stimulated IκBα degradation in human placental explants. Placentas were collected from full-term uncomplicated Cesarean deliveries, as described. The effect of various concentrations of DMA on IκBα degradation was determined in the presence or absence of LPS for 15 min. A graphical summary of the relative densities of IκBα immunoblots determined with ImageJ software is shown.



Supplementary Figure S4. Placental explant viability assay. LDH assay was performed to evaluate the viability of placental explants after exposure to the experimental conditions as shown.