

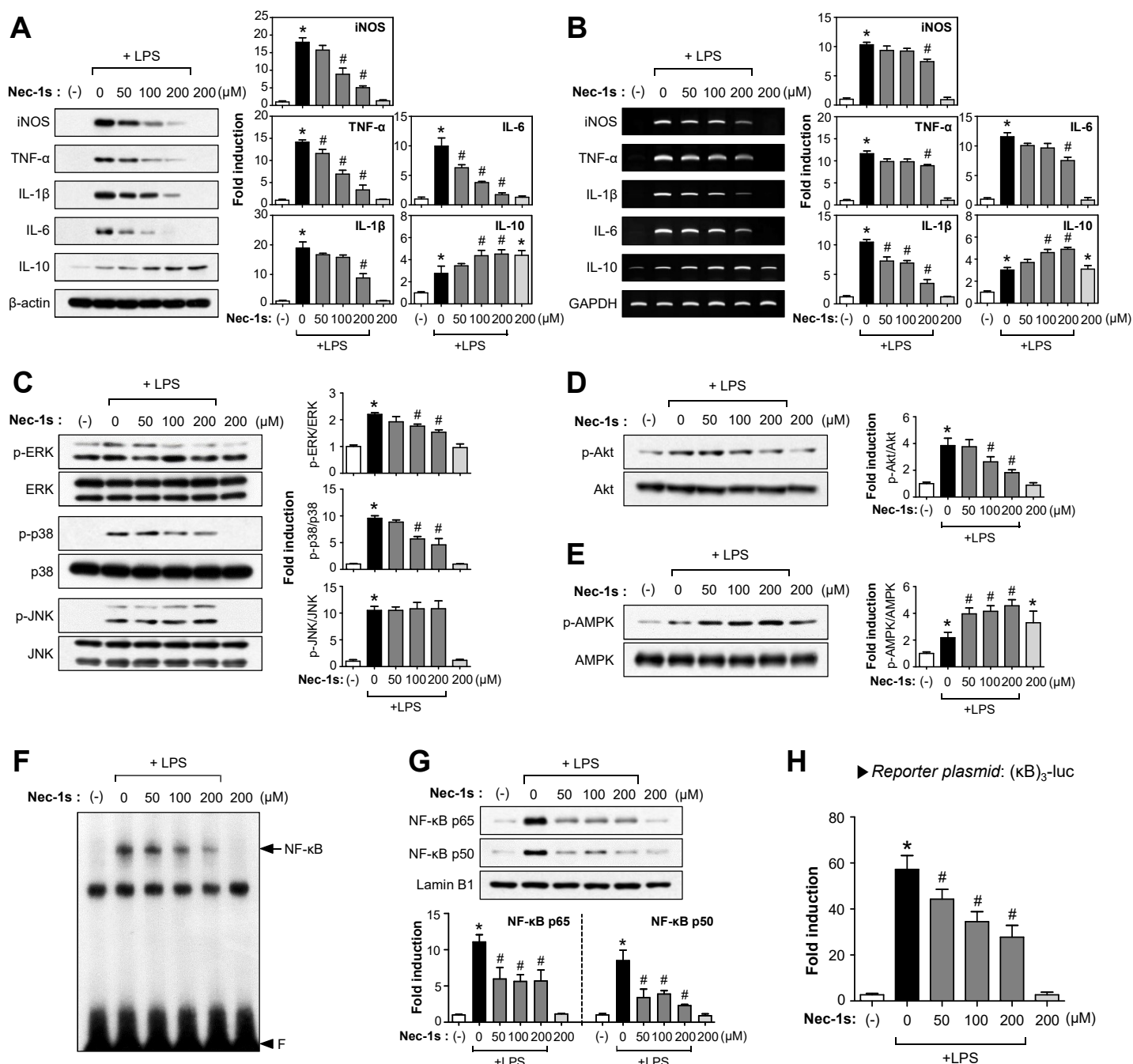


Figure S1. Effect of Nec-1s on inflammatory cytokines and signaling molecules such as MAPKs, Akt, AMPK, and NF- κ B in LPS-stimulated BV2 cells. (A, B) BV2 cells were pretreated with Nec-1s for 1 h and incubated with LPS (100 ng/ml) for 6 h. Western blot analysis (A) and RT-PCR (B) were performed to measure the expression of TNF- α , IL-1 β , IL-6, IL-10. (C)~(E) BV2 cells were pretreated with Nec-1s and incubated with LPS (100 ng/ml) for 1 h. Western blot analysis using antibodies against the phospho- or total forms of ERK, p38, JNK, Akt, AMPK were normalized with respect to the level of each total form and expressed as fold changes relative to the control group. (A)~(E), representative gels are shown in the left panel, and the quantification of three independent experiments is shown in the right panel. (F) EMSA for NF- κ B was performed using nuclear extracts prepared from BV2 cells pretreated with Nec-1s and incubated with LPS (100 ng/ml) for 1 h. (G) Effect of Nec-1s on nuclear translocation of NF- κ B subunits. (H) Transient transfection analysis of (NB)₃-luc reporter gene activity. Data are shown as the mean $\pm$ SEM of three independent experiments. * p < 0.05 vs. control; # p < 0.05 vs. LPS-treated samples.