

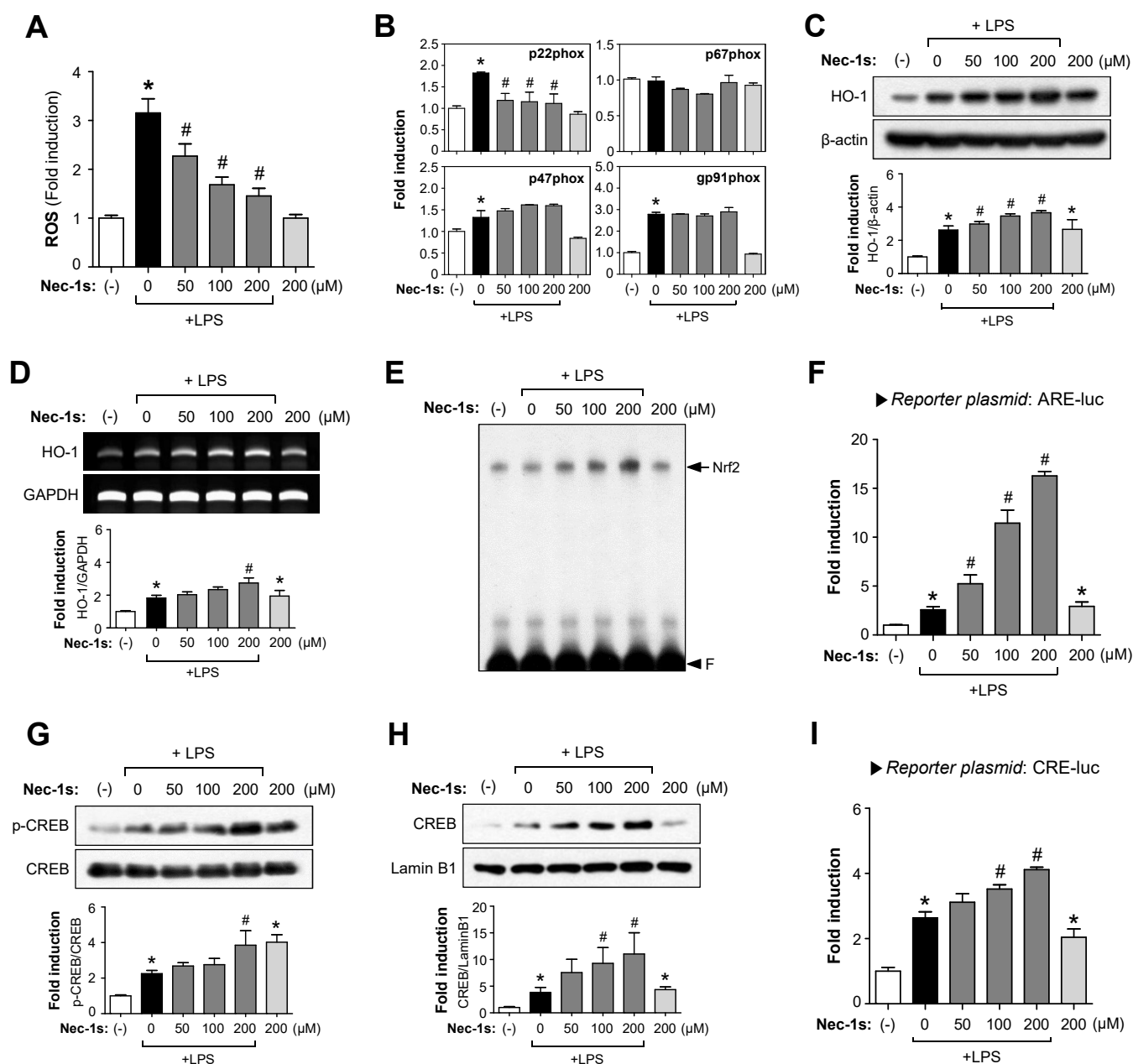


Figure S2. Nec-1s reduced ROS production via suppression of p22^{phox} NADPH oxidase subunit and upregulation of Nrf2/ARE and PKA/CREB signaling. (A) BV2 cells were treated with Nec-1s 1 h prior to LPS stimulation for 8.5 h. Intracellular ROS level was measured using the DCF-DA assay. (B) Quantitative real time PCR to assess mRNA expression of NADPH oxidase subunits (p22^{phox}, p47^{phox}, p67^{phox}, and gp91^{phox}) in BV2 cells. (C, D) Western blot and RT-PCR analysis to determine the effect of Nec-1s on the protein and mRNA expression of HO-1. BV2 cells were treated with Nec-1s for 1 h followed by LPS (100 ng/ml) for 6 h. (E) EMSA for Nrf2 DNA binding activity. BV2 cells pretreated with Nec-1s and incubated with LPS (100 ng/ml) for 1 h. (F) Transient transfection analysis of ARE-luc reporter gene activity. (G, H) Western blot analysis for p-CREB and total CREB was performed using cell lysates and nuclear extracts from BV2 cells, respectively. (I) Transient transfection analysis of CRE-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.