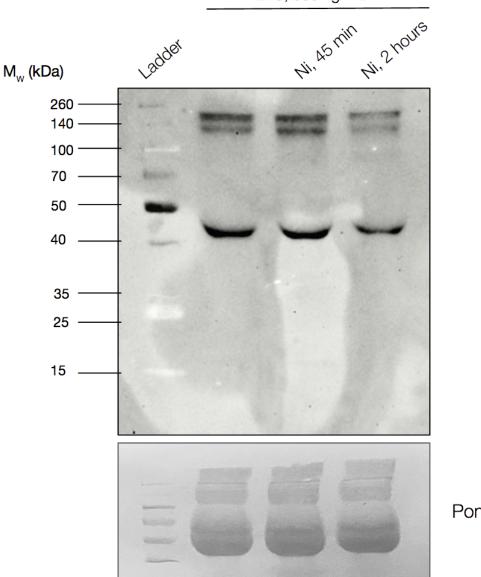


BLUE: 1st treatment RED: 2nd treatment

Supplemental figure S1. The actin cytoskeleton undergoes structural changes in response to LPS and nigericin. Epifluorescence microscopy of J774A.1 murine macrophages in response to 4 hours of LPS stimulation, or 4 hours of LPS stimulation followed by nigericin treatment for 45 minutes or 2 hours. Representative epi-fluorescence images (100X) of J774A.1 macrophages dual-stained with Alexa-Fluor 568-Phalloidin to (red) and DAPI (blue) to co-localize F-actin and the nucleus, respectively. *Scale bar* 40 μm.



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Supplemental figure 2. Concentration of J774A.1 cell culture supernatant did not enable detection of either pro- or cleaved forms of IL- 1 β . Top panel: Western blot for pro- (31 kDa) and mature IL-1 β (17 kDa) in cell culture supernatant of macrophages after 4 hours of LPS stimulation, or 4 hours of LPS stimulation followed by nigericin treatment for 45 minutes or 2 hours. Neither forms of IL-1 β are detected. Meanwhile, three non-specific bands with sizes greater than 40 kDa are observed. **Bottom panel:** Ponceau S staining of The transferred proteins on the PVDF membrane were stained with Ponceau S staining solution (1 mg/ml) to ensure adequate transfer and equal amount of protein loaded on the SDS-PAGE gel.