Reactive oxygen species inhibitors block priming, but not activation of the NLRP3 inflammasome

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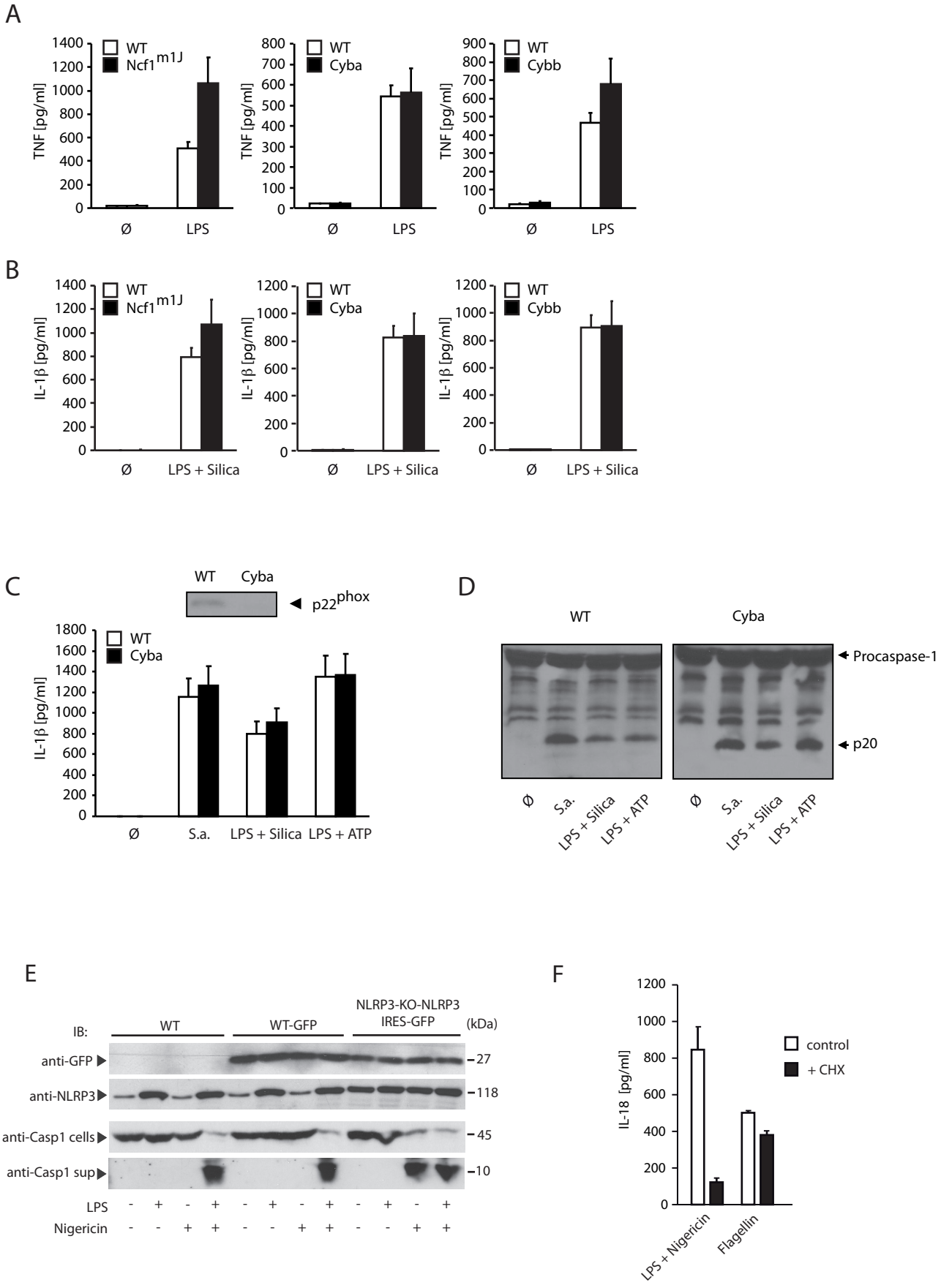
Supplemental Material:

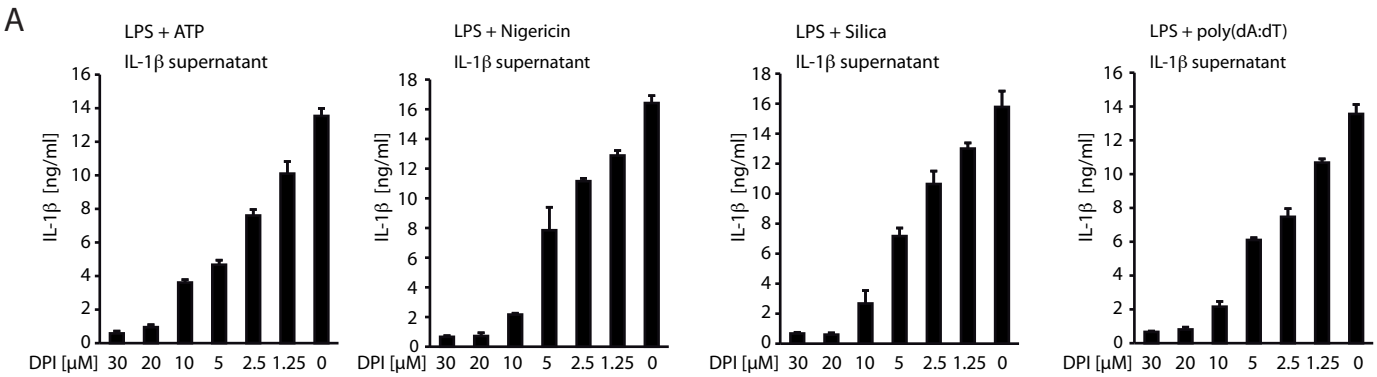
Supplemental Figure 1: **The NADPH oxidase complex is not involved in the activation of the NLRP3 inflammasome.** A-B, Macrophages from wild type mice or mice deficient in the NADPH oxidase subunit $p47^{phox}$ (Ncf1m1J/J), $p91^{phox}$ (Cybb) or $p22^{phox}$ (Cyba) were stimulated with LPS (A) or LPS + Silica (B) for 6 hours. TNF (A) and IL-1b (B) were measured by ELISA. C, LPS-primed macrophages from wild type mice or mice deficient in $p22^{phox}$ (Cyba) were stimulated with *Staphylococcus aurues* supernatant (S.a.), Silica or ATP and IL-1b production was measured by ELISA. The expression of $p22^{phox}$ was evaluated by immunoblotting (insert). D, The activation of caspase-1 was analyzed in extracts prepared from cell and culture supernatants immunoblotted with caspase-1 antibody. Arrows denote procaspase-1 (p45) and its processed p20 subunit. E, Wild type macrophages (WT), macrophages transduced with GFP (WT-GFP) or NLRP3-deficient macrophages transduced with NLRP3-IRES-GFP were stimulated as indicated. Immunoblotting of GFP, NLRP3,

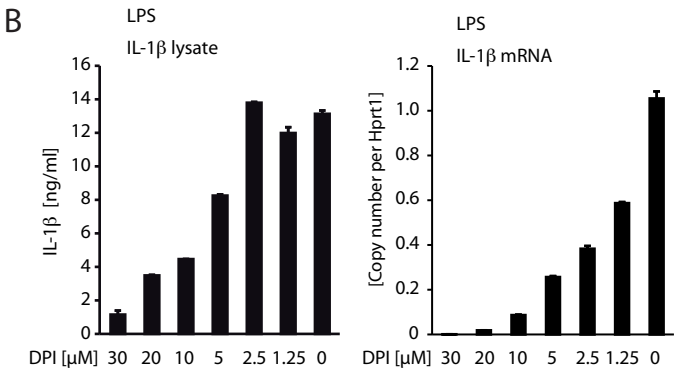
procaspase-1 and cleaved caspase-1 is shown (A). F, Wild type macrophages were stimulated with LPS + Nigericin or flagellin after incubation with 100 ng/ml cyclohexemide (CHX) and IL-18 release was measured by ELISA. Results are representative of three (A-D) or two (E-F) separate experiments.

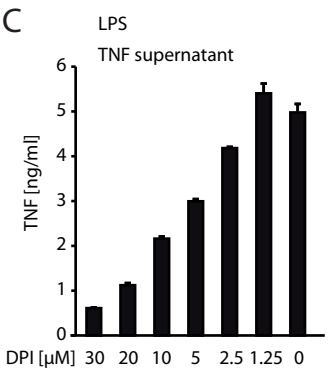
Supplemental Figure 2: Altering the redox state of macrophages inhibits LPS induced cell priming. Wild type macrophages were pretreated with the indicated concentration of DPI (A-C) or NAC (D-G) for 1 h and then stimulated as indicated. IL-1β secretion of LPS-primed (200 ng/ml) cells stimulated with ATP, Nigericin, Silica, or poly(dA:dT) (A and D) is shown. B and E, IL-1β protein concentration and mRNA level in cell lysates is depicted. C and F, TNF mRNA and NLRP3 mRNA expression of LPS-stimulated cells is shown. G, Wild type or NLRP3-deficient macrophages reconstituted with NLRP3 were stimulated as indicated and caspase-1 cleavage was assessed. Data from one representative experiment out of three are shown (A-G).

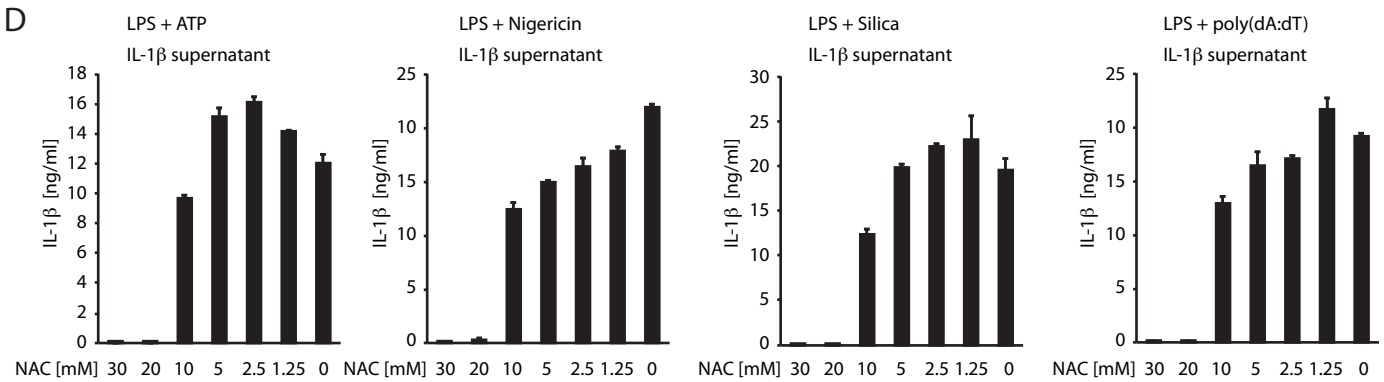


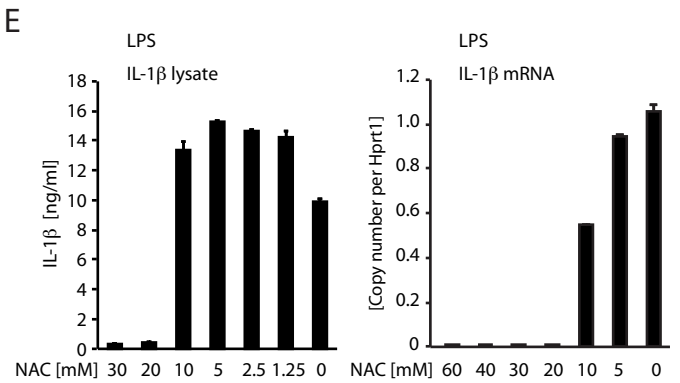
Supplemental Figure 1

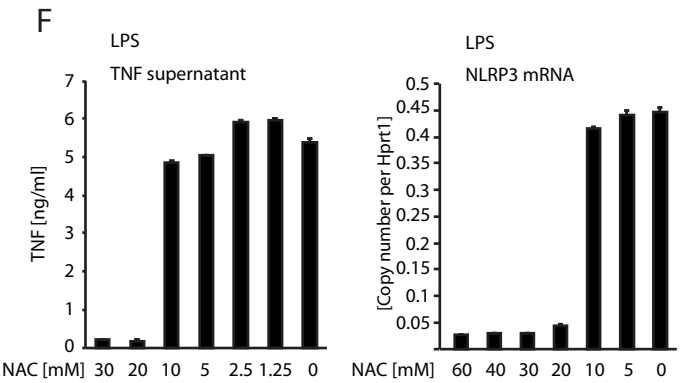


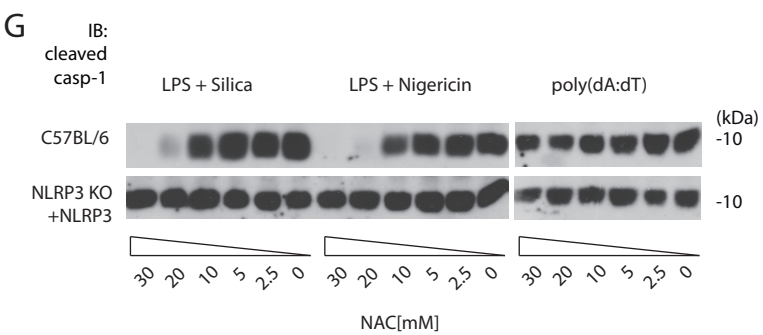












Supplemental Figure 2