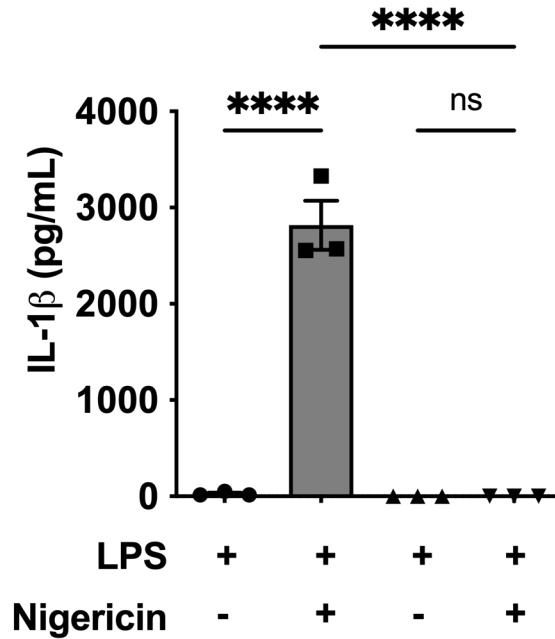


**Cell Reports, Volume 43**

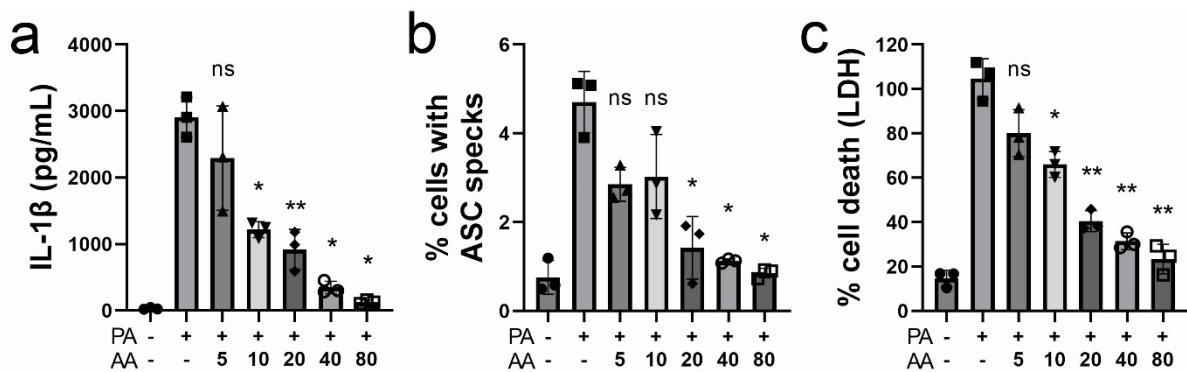
**Supplemental information**

**Arachidonic acid inhibition of the NLRP3  
inflammasome is a mechanism to explain  
the anti-inflammatory effects of fasting**

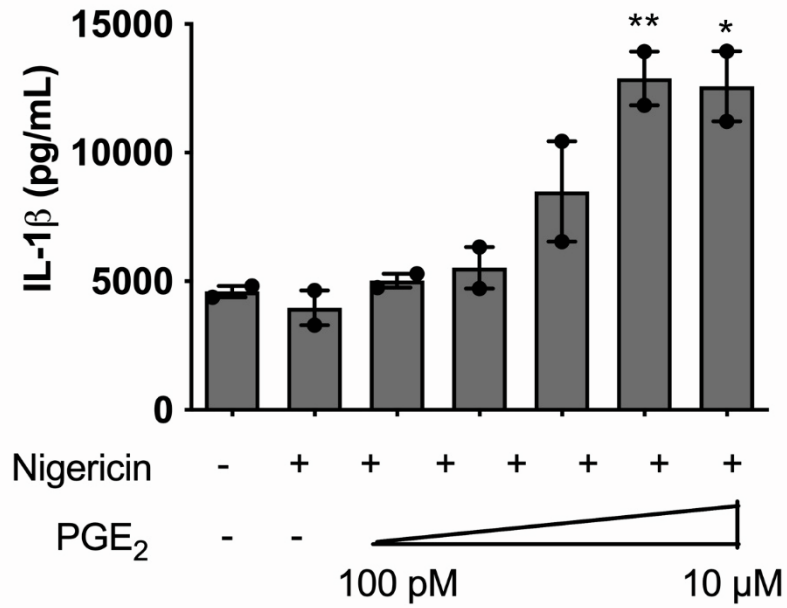
**Milton Pereira, Jonathan Liang, Joy Edwards-Hicks, Allison M. Meadows, Christine Hinz, Sonia Liggi, Matthias Heprich, Jonathan M. Mudry, Kim Han, Julian L. Griffin, Iain Fraser, Michael N. Sack, Christoph Hess, and Clare E. Bryant**



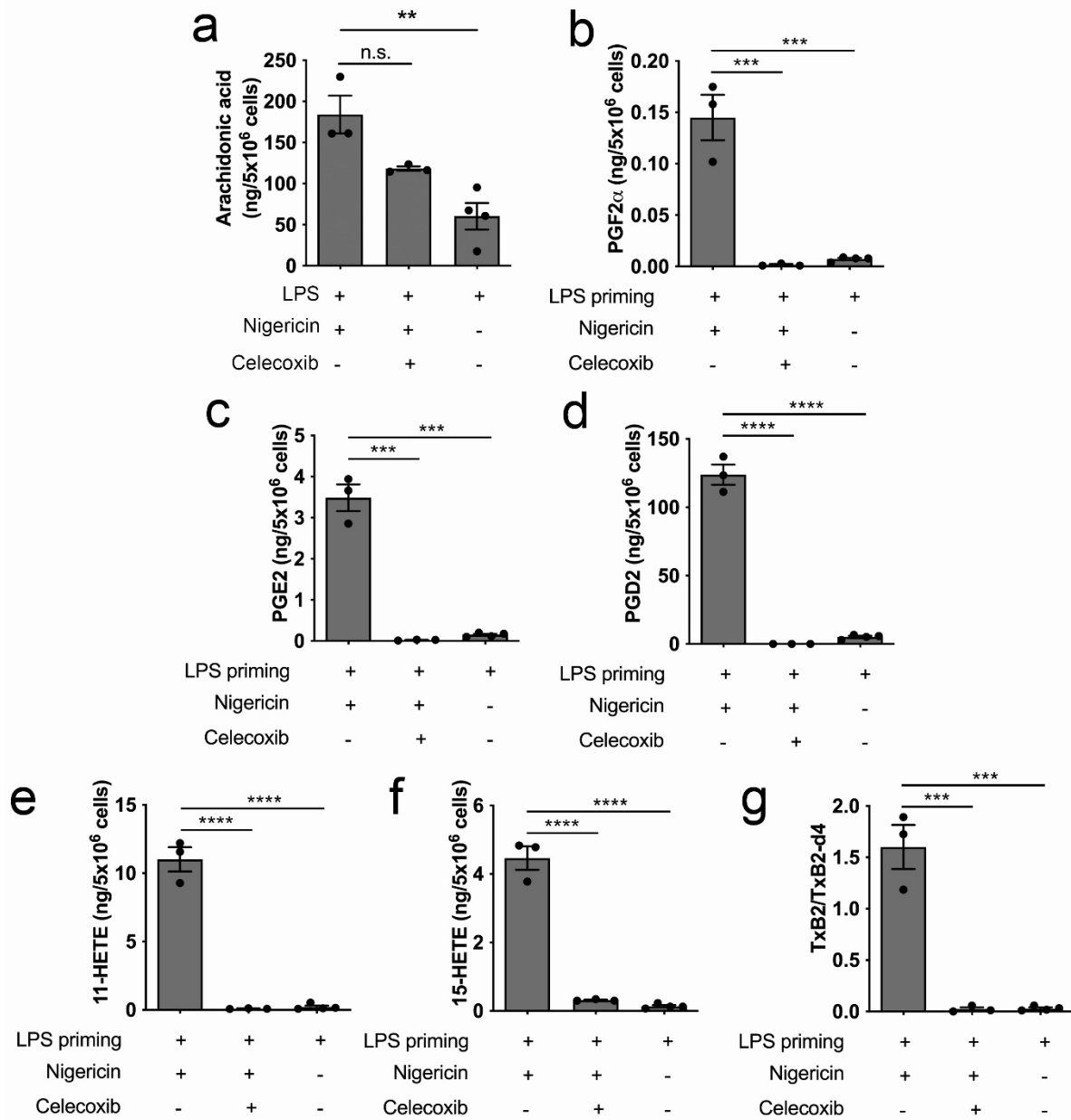
**Figure S1: IL-1 $\beta$  production in nigericin-treated WT and *Nlrp3*<sup>-/-</sup> BMDMs, related to Figure 2.** IL-1 $\beta$  present in culture supernatants of LPS-primed (200 ng/mL, 3 hours) WT and *Nlrp3*<sup>-/-</sup> BMDMs stimulated with nigericin (10  $\mu$ M, one hour). Data are representative of three independent experiments, \*\*\*\* p < 0.0001 (one-way analysis of variance with Tukey's multiple comparison test). Data from three independent experiments (mean and s.e.m.).



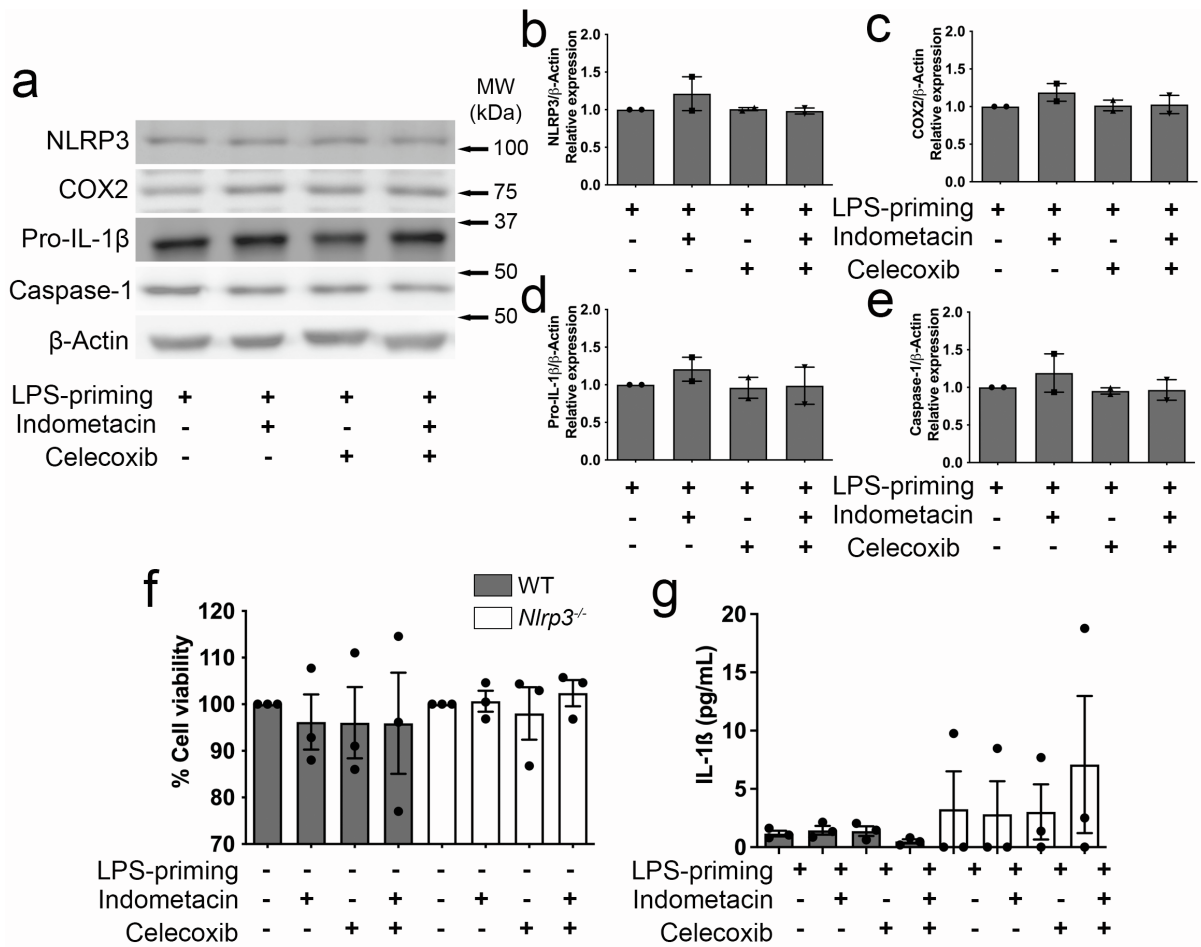
**Figure S2: Dose-dependent inhibition of NLRP3 inflammasome activity by arachidonic acid, related to Fig. 3.** THP-1 cells were differentiated with PMA (200 ng/mL) for 24 hours, rested for 24 hours, then primed with Pam3CSK4 (200 ng/mL) for 4 hours. Cells were then treated with AA at the indicated concentrations ( $\mu$ M) for 30 minutes prior to addition of PA (500  $\mu$ M, BSA-conjugated). After 24 hours, supernatant was collected and cells were fixed and immunostained for ASC. (a) IL-1 $\beta$  in supernatant, (b) cells with ASC specks, and (c) cell death by LDH release assay were measured. Data are representative of three independent experiments, \* p < 0.05, \*\* p < 0.01 (one-way analysis of variance with Tukey's multiple comparison test). n.s., not significant.



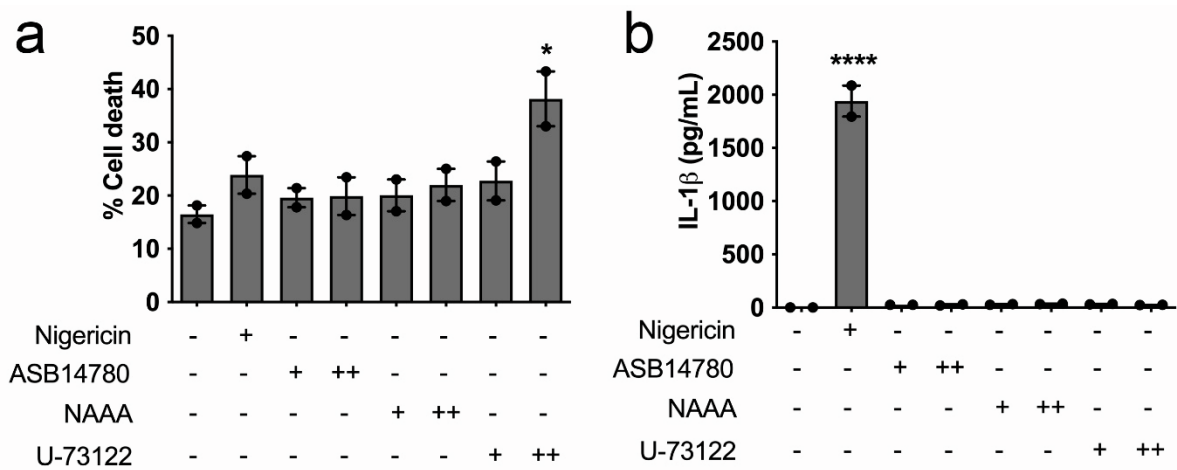
**Figure S3: Effects of PGE<sub>2</sub> on NLRP3 activity, related to Fig. 4.** Increasing concentrations of PGE<sub>2</sub> were added during LPS-priming (200 ng/mL for 3 hours) of WT BMDMs and then stimulated with 10 μM nigericin in presence of PGE<sub>2</sub> and IL-1β in the supernatant quantified. \* p < 0.05, \*\* p < 0.01 (one-way analysis of variance with Tukey's multiple comparison test). Data from two independent experiments (mean and s.e.m.).



**Figure S4: Celecoxib abolishes the production of COX-derived eicosanoids.** (a-g) LC/DTIM-MS quantification of arachidonic acid (a), PGF2 $\alpha$  (b), PGE2 (c), PGD2 (d), 11-HETE (e), 15-HETE (f), and TxB2 (g) after one-hour treatment of LPS-primed (200 ng mL<sup>-1</sup> for 3 hours) WT BMDMs with 10  $\mu$ M nigericin with or without 10  $\mu$ M celecoxib. \*\*\* p < 0.001, \*\*\*\* p < 0.0001 (one-way analysis of variance with Tukey's multiple comparison test). Data from three independent experiments (mean and s.e.m.).



**Figure S5: COX inhibition does not impact the expression of NLRP3-related proteins.** (a-e) Expression and  $\beta$ -Actin-adjusted relative densitometry of NLRP3 (a, b), COX-2 (a, c), pro-IL-1 $\beta$  (a, d), and caspase-1 (a, e). (f-g) Cellular viability (f) and IL-1 $\beta$  production (g) in LPS-primed (200 ng/mL, 3 hours) WT and *Nlrp3*<sup>-/-</sup> BMDMs during one-hour incubation with 100  $\mu$ M indomethacin, 10  $\mu$ M celecoxib, or both. Dashed line represents the assay detection limit. (a) Image is representative of three independent experiments. (b-g) Data from three independent experiments (mean and s.e.m.).



**Figure S6: Effects of PLA<sub>2</sub> and PLC inhibitors on cell death and IL-1 $\beta$  production, related to Fig. 6.** Cell death (a) and IL-1 $\beta$  production (b) in LPS-primed (200 ng/mL for 3 hours) WT BMDMs in response one-hour stimulation with 1  $\mu$ M (+) or 10  $\mu$ M (++) of PLA<sub>2</sub> inhibitors ASB1414780 and NAAA, or PLC inhibitor U-73122. \*  $p < 0.05$ , \*\*\*\*  $p < 0.0001$  in comparison to unstimulated control (one-way analysis of variance with Tukey's multiple comparison test). Data from two independent experiments (mean and s.e.m.).