

## Supplementary Information

### **The vasoconstrictor adenosine 5'-tetrphosphate is a danger signal that induces IL-1 $\beta$**

Judith Bockstiegel<sup>1</sup>, Jonas Engelhardt<sup>1</sup>, Mirjam Schuchardt<sup>2,3</sup>, Markus Tölle<sup>2</sup>,

Günther Weindl<sup>1\*</sup>

<sup>1</sup> Pharmaceutical Institute, Pharmacology and Toxicology section, University of Bonn,  
Gerhard-Domagk-Str. 3, 53121 Bonn, Germany.

<sup>2</sup> Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and  
Humboldt Universität zu Berlin, Department of Nephrology and Medical Intensive Care,  
Hindenburgdamm 30, 12203 Berlin, Germany.

<sup>3</sup> Medical School Berlin, Faculty of Medicine, Rüdesheimer Straße 50, 14513 Berlin,  
Germany.

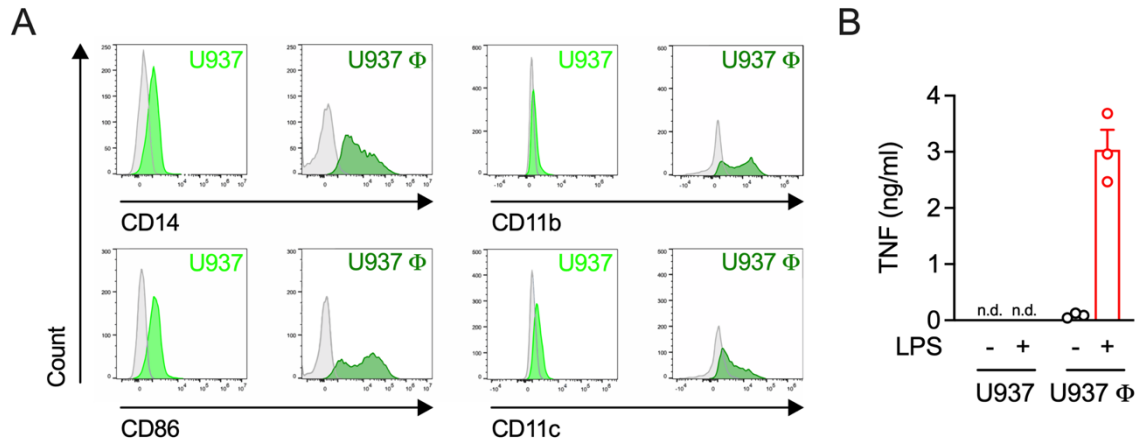
\* Corresponding author: Dr. Günther Weindl, Pharmacology and Toxicology section,  
Pharmaceutical Institute, University of Bonn, 53121 Bonn, Germany. Phone: +49 228  
739103, E-mail address: guenther.weindl@uni-bonn.de, ORCID: 0000-0002-4493-7597

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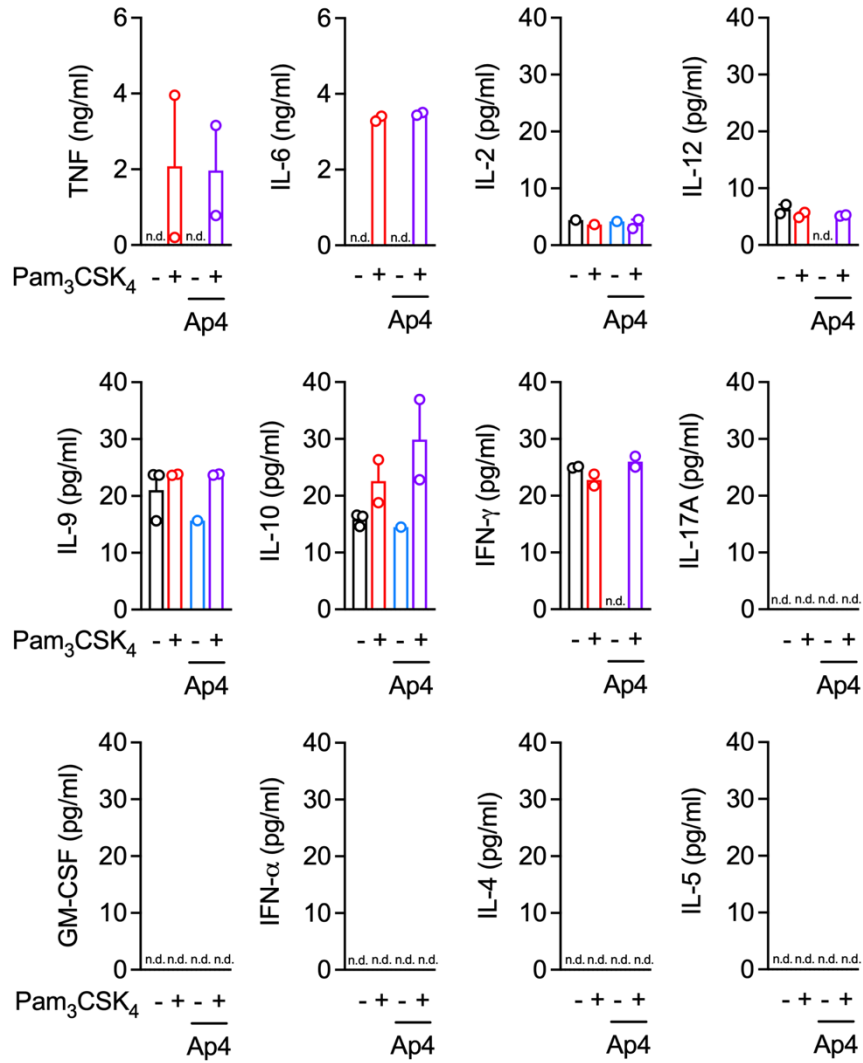
Supplementary Figures 1 to 7

Uncropped images of Western blots in Supplementary Figure 7

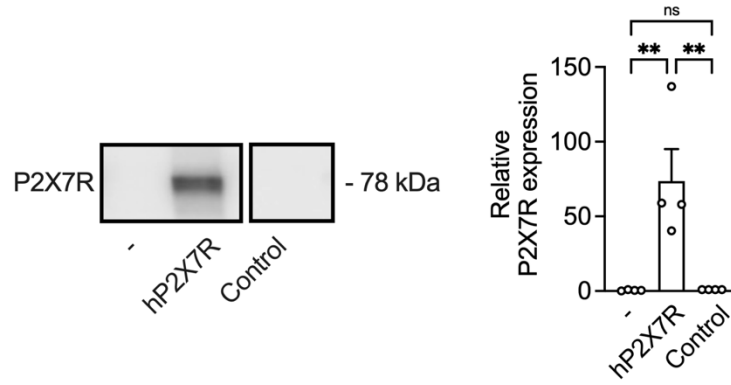
## Supplementary Figures



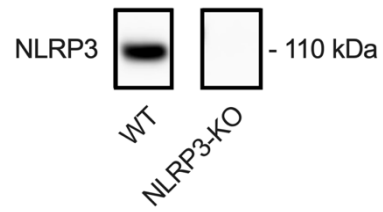
**Fig. S1. Macrophages generated from U937 promonocytes express typical differentiation markers and respond to LPS.** (A) U937 cells were stimulated for 72 h without or with 50 ng/ml PMA. This was followed by a 24-hour recovery period in PMA-free medium. Flow cytometry was used to measure the change in surface markers CD14, CD11b, CD11c and CD86 (coloured curves) compared to the respective fluorescence minus one (FMO) controls (grey curves). The graphs are representative of two independent experiments. Following the recovery phase, cells were stimulated with LPS *E. coli* (10 ng/ml). TNF secretion in the cell culture supernatants was determined after 4 h by ELISA. Mean  $\pm$  SEM (n = 3).



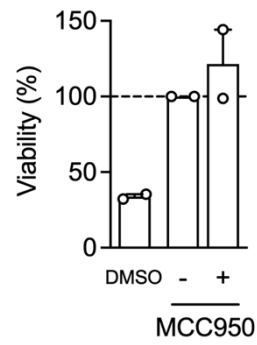
**Fig. S2. Ap4 does not modulate cytokine release in unprimed and primed PBMCs.** PBMCs were primed without or with Pam<sub>3</sub>CSK<sub>4</sub> (1  $\mu$ g/ml) for 3 h and then stimulated with Ap4 (5 mM) for 3 h. Cell culture supernatants were analyzed for cytokine concentrations by multiplex assay using flow cytometry. n.d. = not detected. Mean  $\pm$  SEM (n = 2).



**Fig. S3. Transfection of HEK293 cells with P2X7 receptor.** HEK293 cells were transfected with the hP2X7 receptor (pUNO1-hP2RX7) or the control plasmid (pUNO-mcs). P2X7 receptor protein levels from cell lysates of transfected and untransfected HEK293 cells were determined by Western blot analysis. The data represent four independent experiments. P2X7 receptor protein levels were normalized to the respective total protein levels and the respective values were referred to the control (control denoted by the value 1). Mean  $\pm$  SEM ( $n = 4$ ). One-way ANOVA followed by Tukey's post-test, ns  $\geq 0.05$ , \*\* $P \leq 0.01$ .

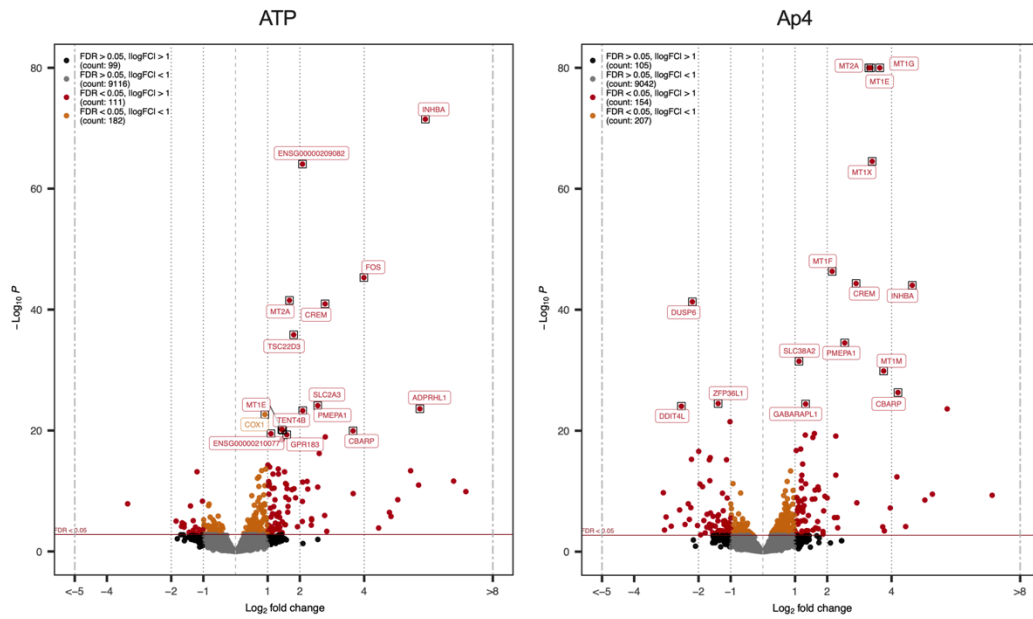


**Fig. S4. NLRP3 protein levels of THP-1 macrophages and NLRP3-KO THP-1 macrophages.** NLRP3 protein levels from cell lysates of THP-1 macrophages (WT) and NLRP3-KO THP-1 macrophages (NLRP3-KO) were determined by Western blot analysis. The data represent four independent experiments.

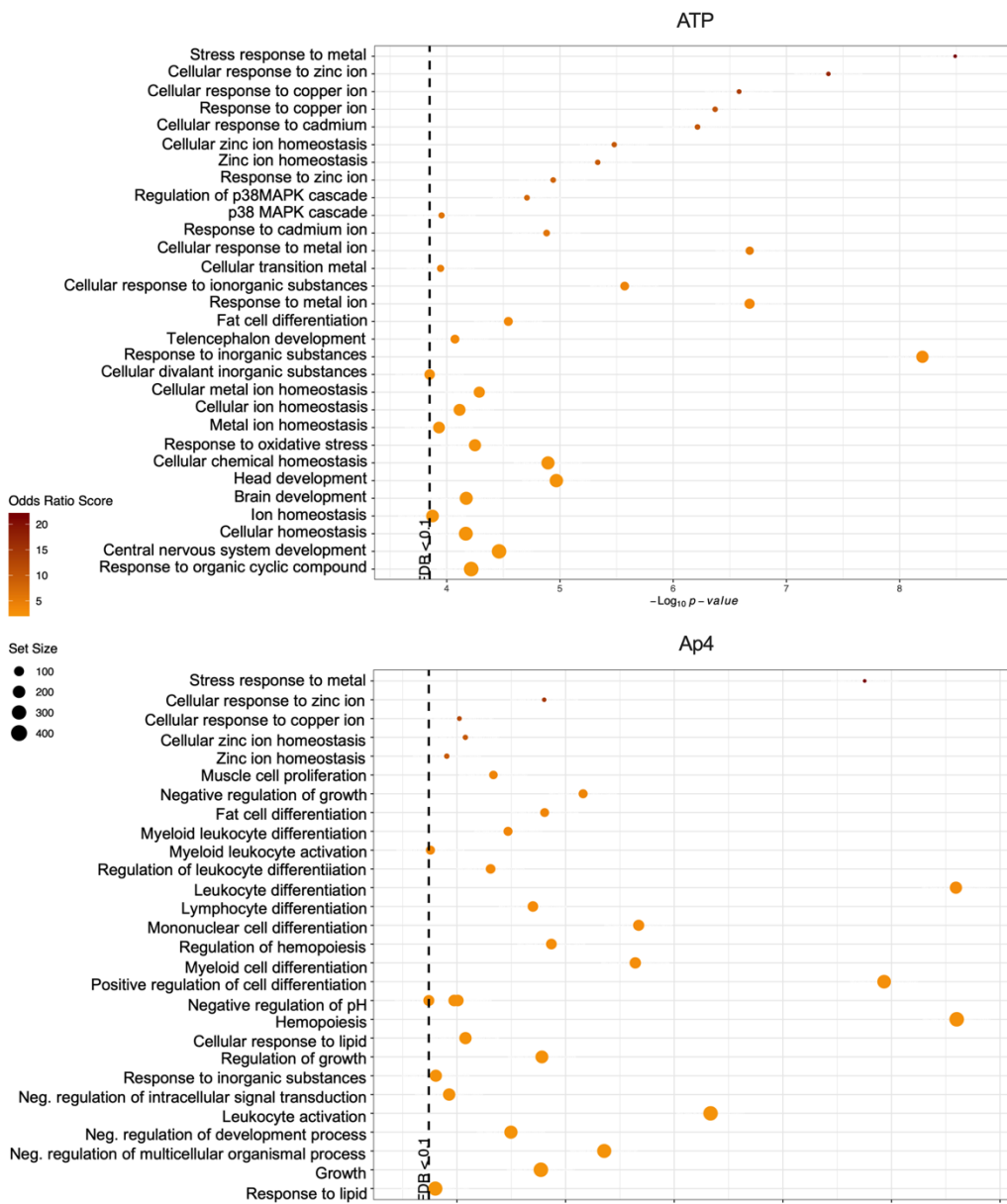


**Fig. S5. Cell viability of U937 macrophages is not affected by MCC950.** U937 macrophages were stimulated with MCC950 (40  $\mu$ M). Cell viability was determined after 7 h by MTT assay, using DMSO (10%, v/v) as cytotoxic control. Viability was referred to the unstimulated control (control set to 100%). Mean  $\pm$  SEM (n = 2).

A



B

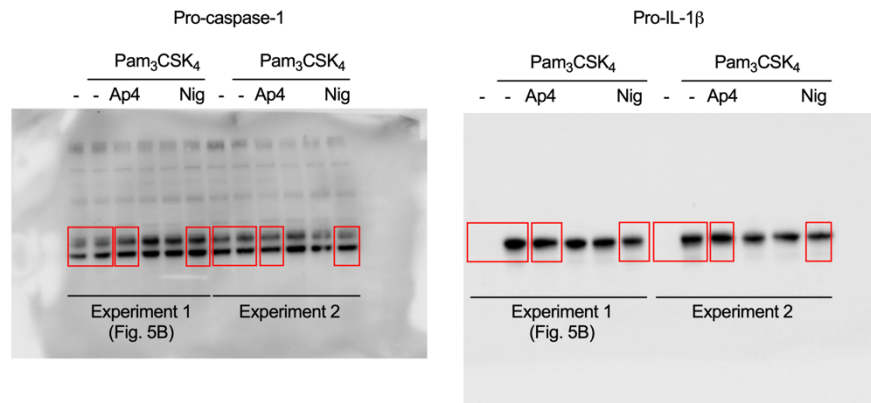


**Fig. S6. ATP and Ap4 differentially regulate gene expression.** THP-1 macrophages were stimulated for 3 h without or with Ap4 (5 mM) or ATP (5 mM). Afterwards RNA sequencing was performed. (A) Volcano plot of all 9508 identified genes indicate differentially expressed genes after treatment with ATP (left) or Ap4 (right) compared to control (RPMI). The negative  $\log_{10}$  transformed p-values (y-axis) were plotted against the average  $\log_2$ -fold change (x-axis) in gene expression. Transcripts with a p-value  $< 0.5$  were classified as statistically significant (red lines indicate cut-off values for p-value). Differentially expressed gene (DEGs) defined as genes with a log two-fold change value of less than -1 or more than 1. Significantly differentially expressed transcripts are denoted in red. (B) Dot plot of gene ontology (GO) enrichment analysis showing enriched biological processes, sorted by absolute score. Colors indicate the odds ratio and dots size represent the number of DEG in the given pathway.

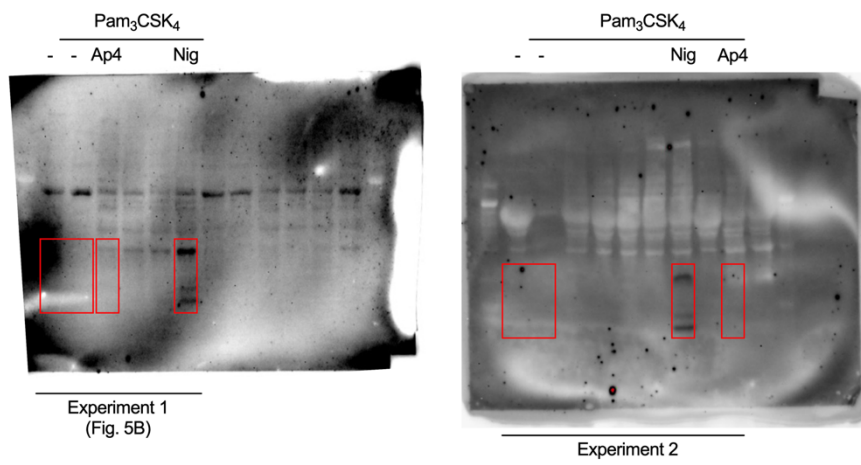


A

THP-1 Mφ

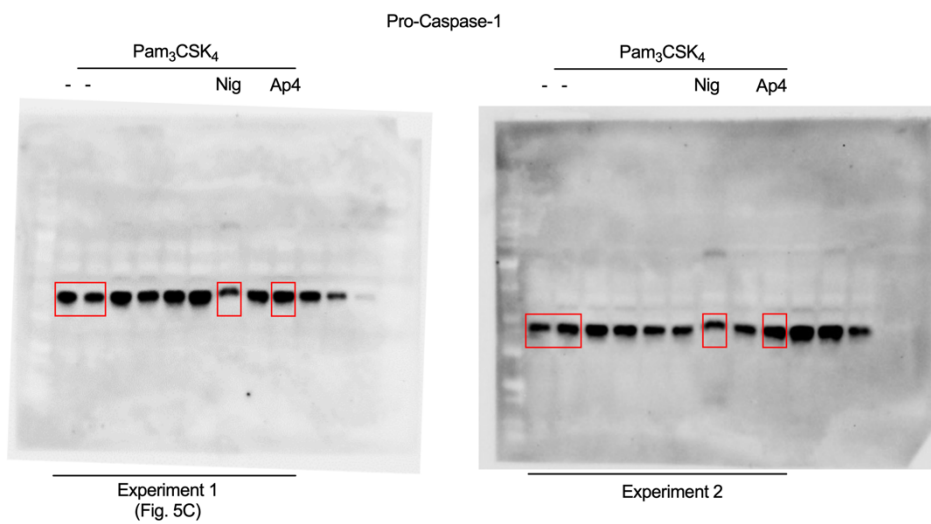


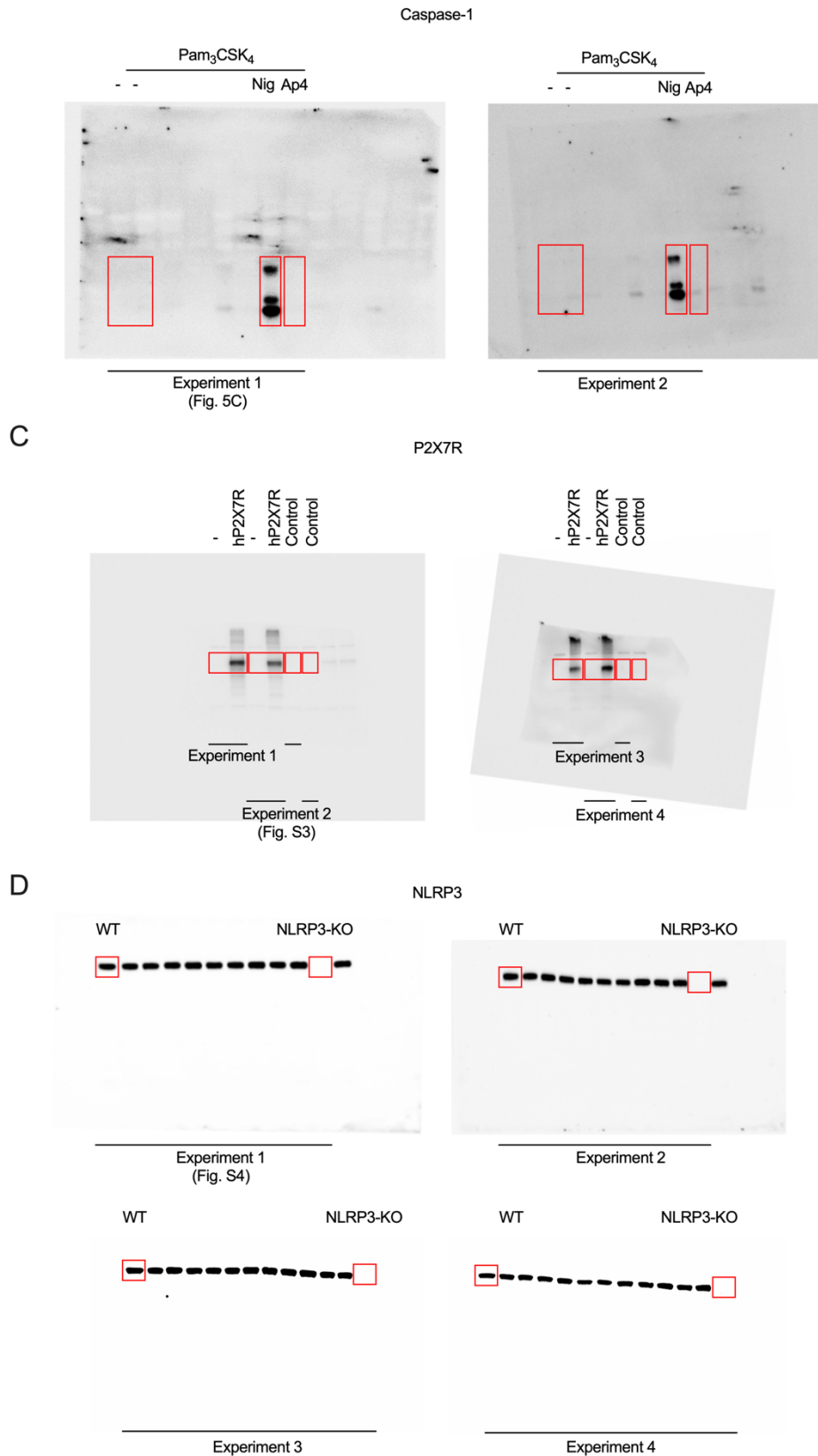
Caspase-1



B

THP-1 Monocytes





**Fig. S7. Uncropped images of the original western blots.** (A) Complete western blots of pro-caspase-1, pro-IL-1 $\beta$  and caspase-1 shown in Fig. 5B. (B) Complete western blots of pro-caspase-1 and caspase-1 shown in Fig. 5C. (C) Complete western blots of P2X7R shown in Fig. S3. (D) Complete western blots of NLRP3 shown in Fig. S4.