

Supplementary Materials

Protein-bound uremic toxins induce reactive oxygen species-dependent and inflammasome-mediated IL-1 β production in kidney proximal tubule cells

Milos Mihajlovic¹, Merle M. Krebber², Yi Yang¹, Sabbir Ahmed¹, Valeria Lozovanu¹, Daria Andreeva¹, Marianne C. Verhaar² and Rosalinde Masereeuw^{1,*}

¹Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, 3584 CG Utrecht, The Netherlands

²Department Nephrology and Hypertension, University Medical Center Utrecht, 3508 GA Utrecht, The Netherlands

*Correspondence: Prof. Dr. Rosalinde Masereeuw; e-mail: r.masereeuw@uu.nl

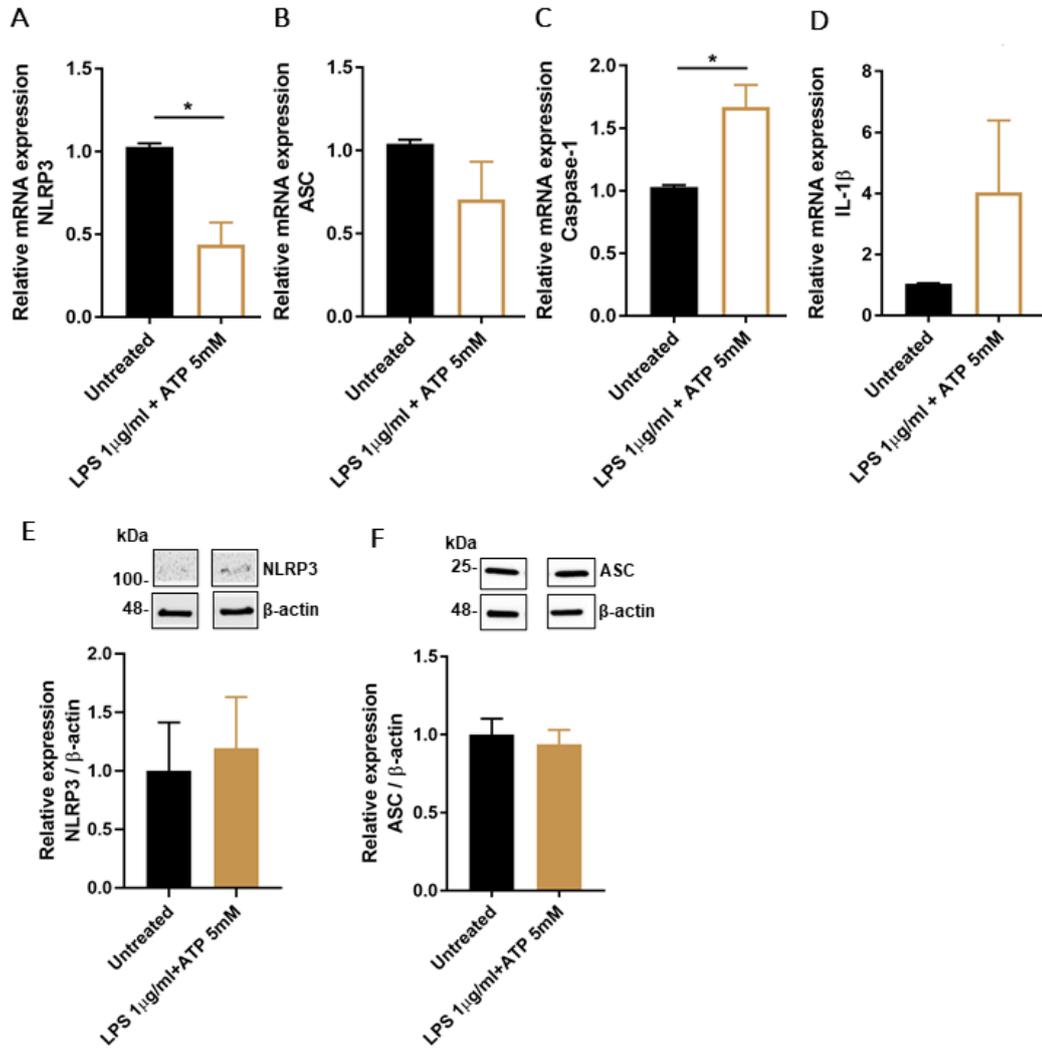


Figure S1. Effect of LPS/ATP treatment on expression of NLRP3 inflammasome-related components in ciPTEC-OAT1. Relative mRNA expression of **A**) NLRP3, **B**) ASC, **C**) caspase-1 and **D**) IL-1 β upon exposure to LPS (1 μ g/ml; 24 h) and ATP (5 mM; 30 min), compared to control (untreated ciPTEC-OAT1). Representative Western blot and quantification of relative expression of **E**) NLRP3 and **F**) ASC in ciPTEC-OAT1 following exposure to LPS (1 μ g/ml; 24 h) and ATP (5 mM; 30 min); normalized to β -actin. Data are derived from three independent experiments performed in triplicate and expressed as mean \pm SEM. * $p < 0.05$ (Unpaired t test).

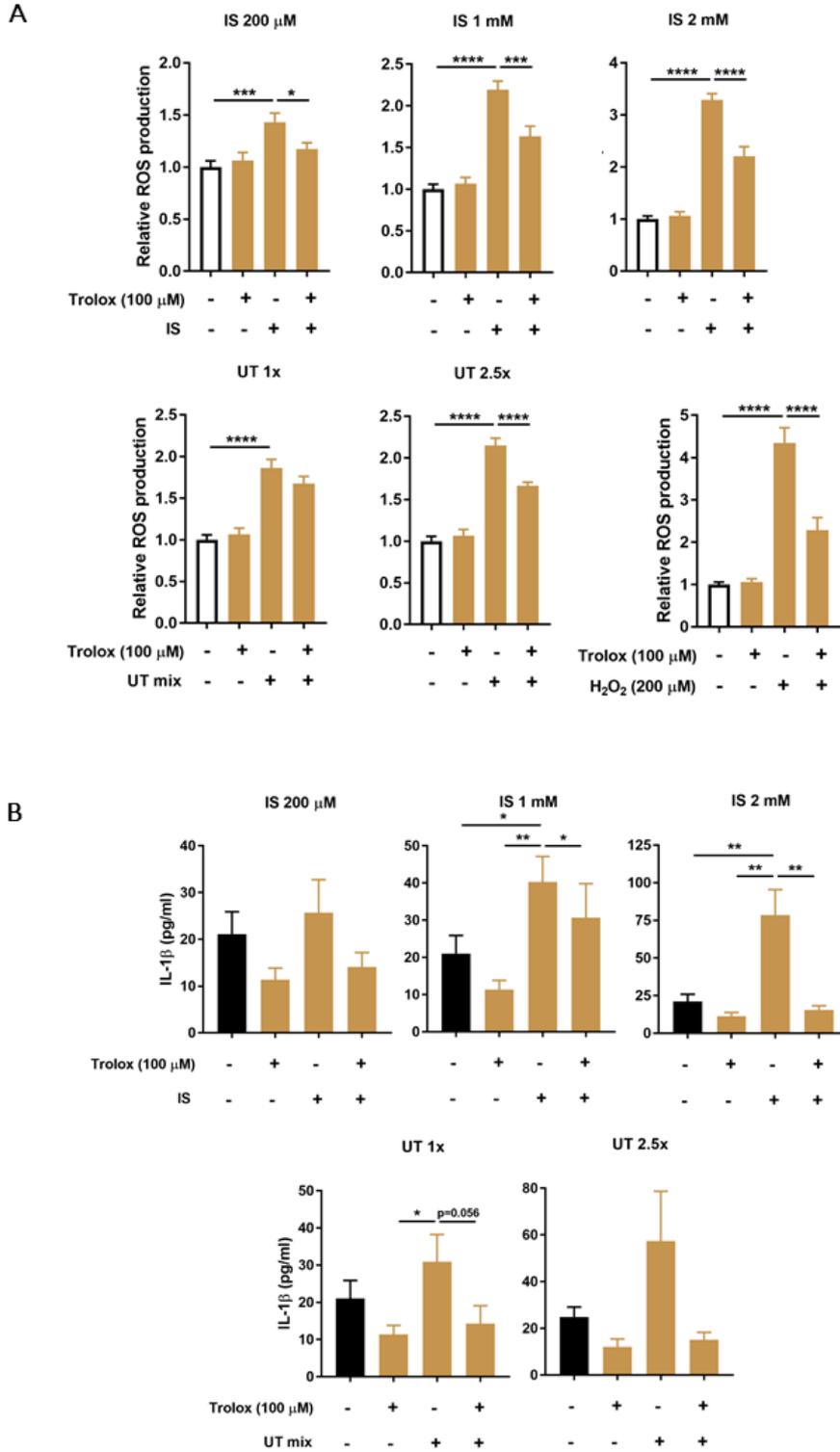


Figure S2. Effect of PBUTs-induced oxidative stress and antioxidant Trolox on IL-1 β production in ciPTEC-OAT1. **A)** Relative intracellular ROS production in ciPTEC-OAT1 after 2 h exposure to IS (200 μ M, 1 mM, 2 mM), UT mix (1x, 2.5x) and H₂O₂ (200 μ M, positive control), in the absence or presence of ROS inhibitor Trolox (100 μ M). **B)** IL-1 β secreted levels (pg/ml) by ciPTEC-OAT1 upon 24 h treatment with IS (200 μ M, 1 mM, 2 mM) and UT mix (1x, 2.5x), in the absence or presence of Trolox (100 μ M). Data are derived from three independent experiments performed in triplicate and expressed as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (One-way ANOVA followed by Dunnett's multiple comparison test, using PBUTs treated cells as a control).

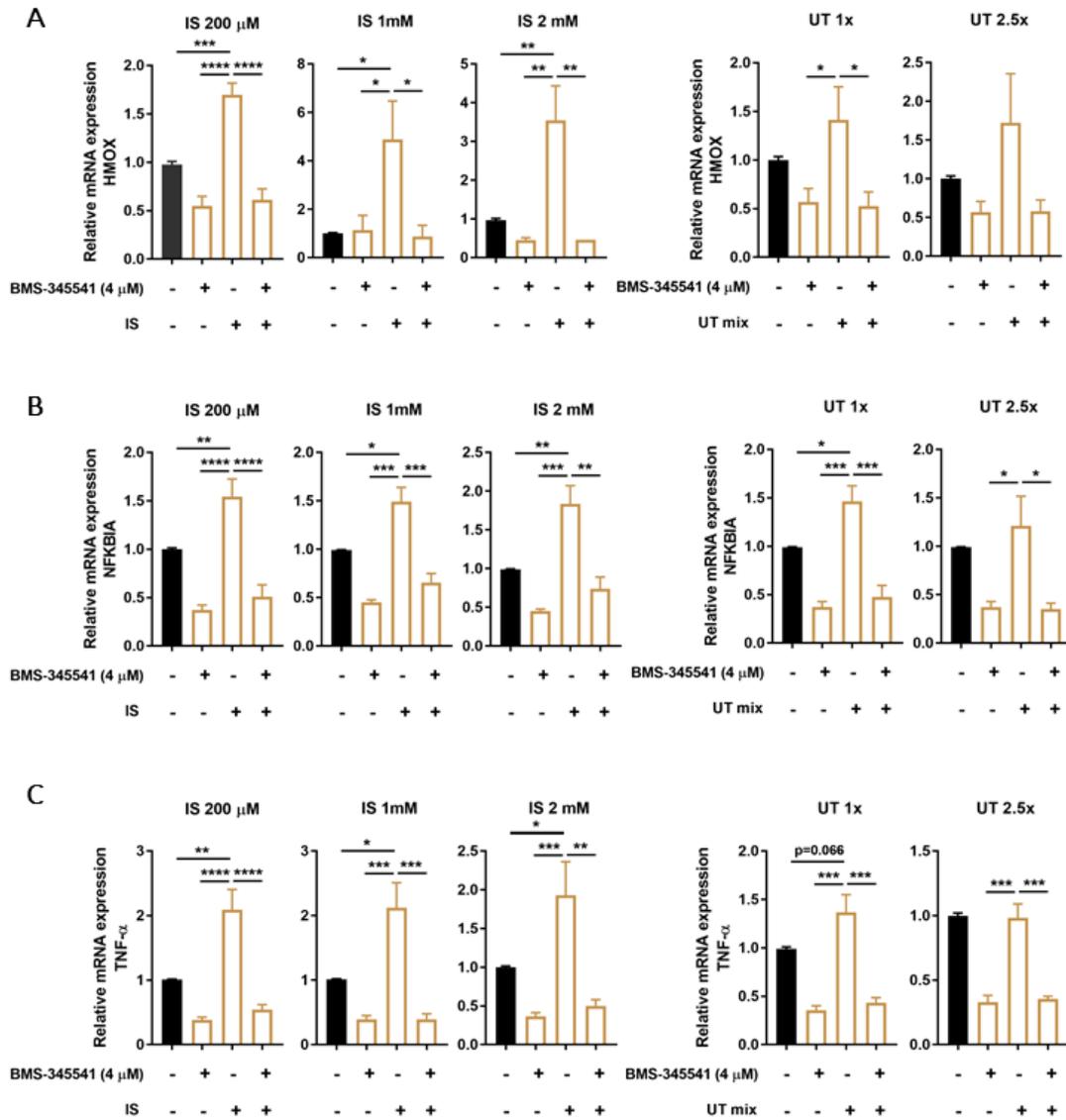


Figure S3. Effect of PBUTs on NF- κ B target genes expression levels in ciPTEC-OAT1. Relative mRNA expression of **A**) heme oxygenase 1 (*HMOX1*), **B**) NF κ B inhibitor alpha (*NFKBIA*) and **C**) TNF- α (*TNF*) upon 4 h exposure to either IS (200 μ M, 1 mM, 2 mM) or UT mix (1x, 2.5x), in the absence or presence of specific IKK inhibitor BMS-345541 (4 μ M) and compared to control (untreated ciPTEC-OAT1). Data are derived from three independent experiments performed in duplicate and expressed as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (One-way ANOVA followed by Dunnett's multiple comparison test, using PBUTs treated cells as a control).