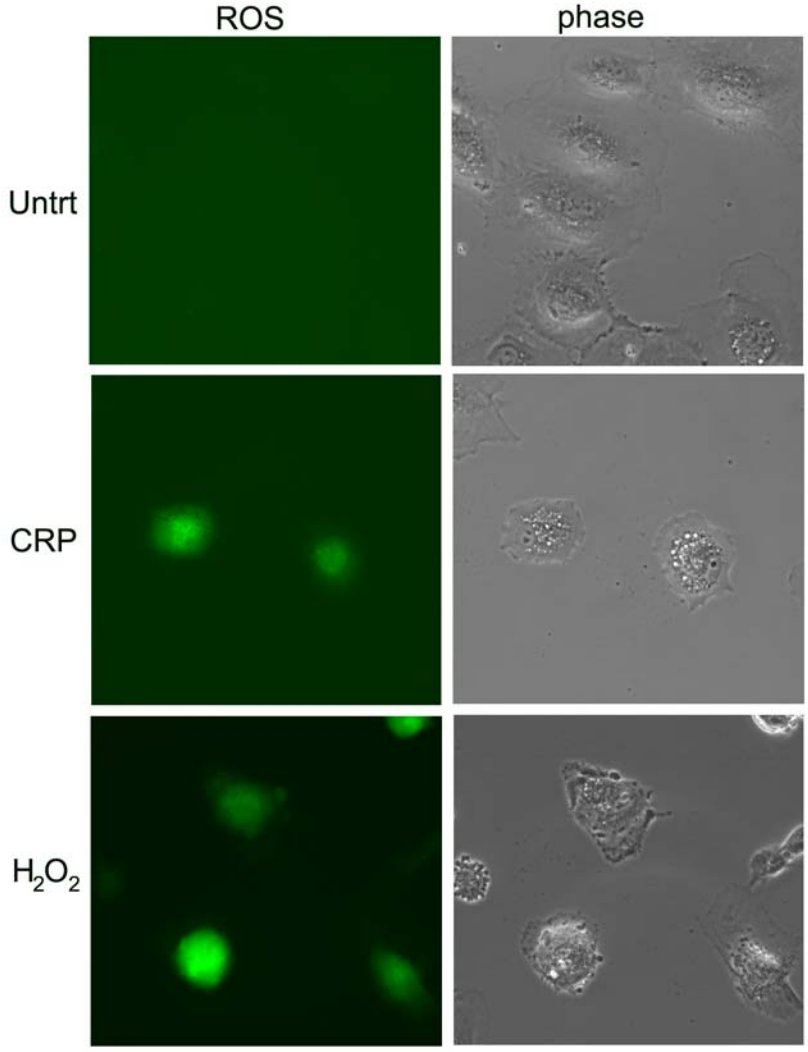
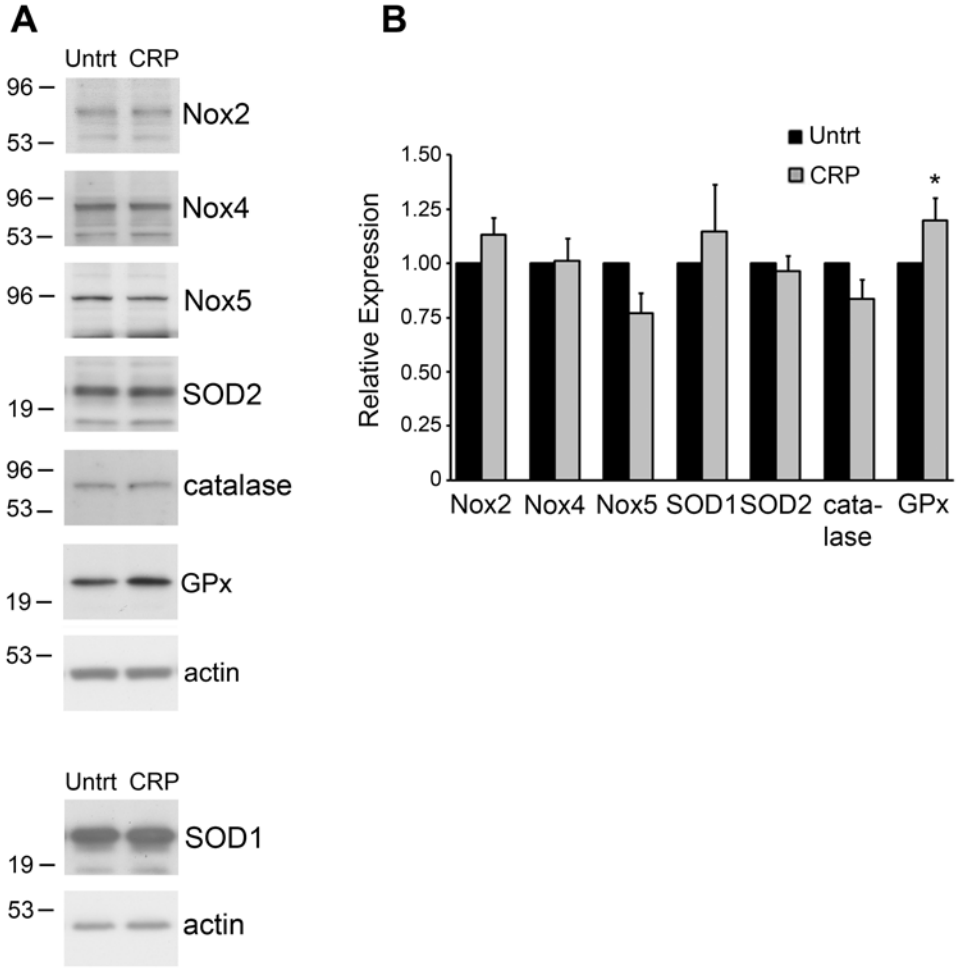
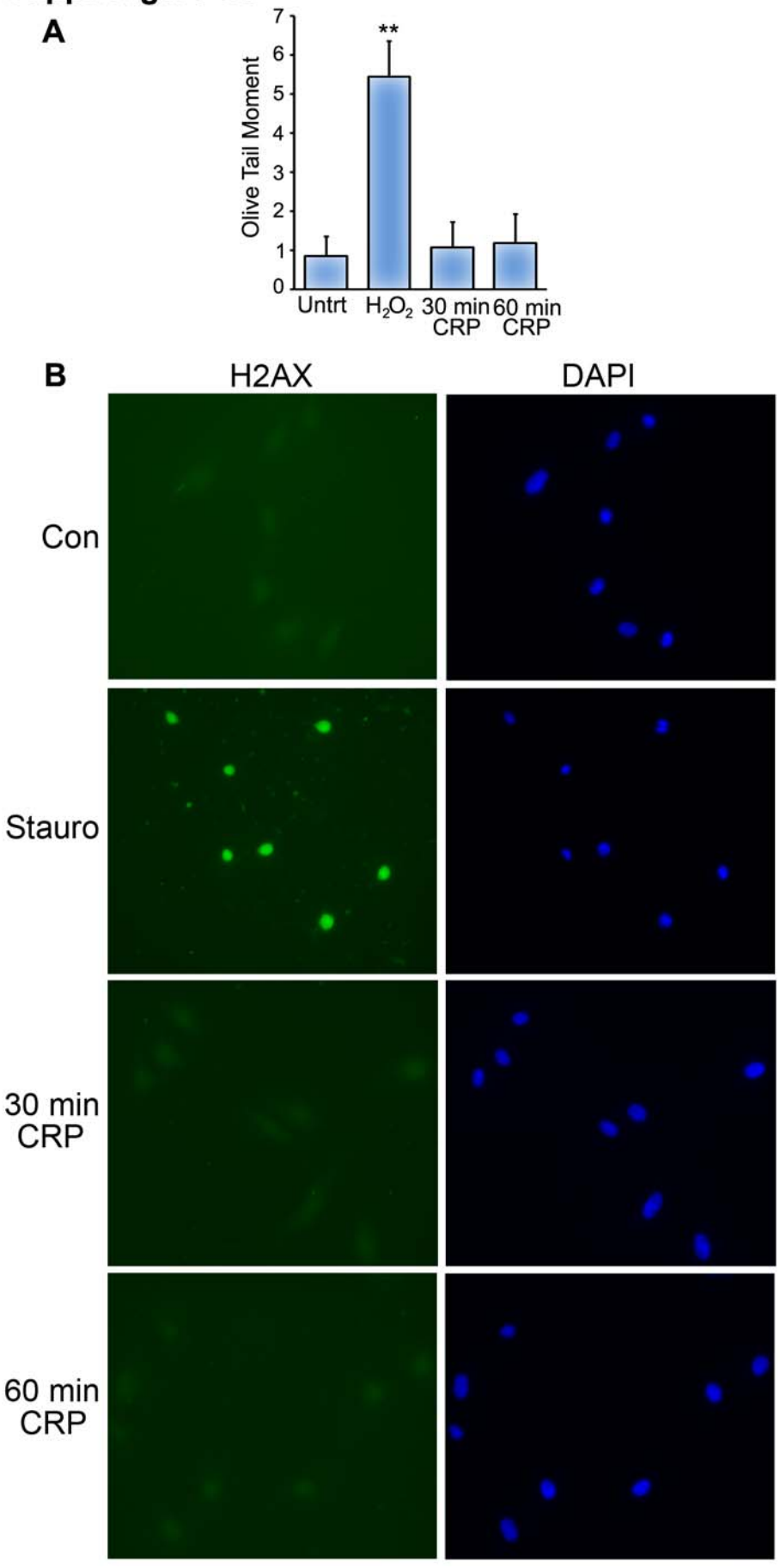
Suppl. Figure S1



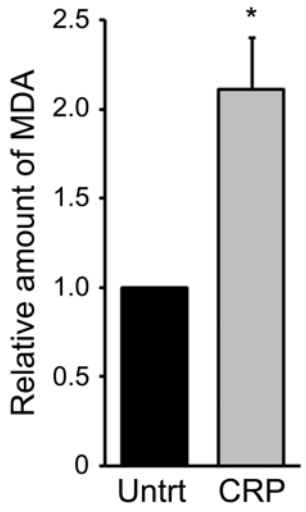
Suppl. Figure S2



Suppl. Figure S3



Suppl. Figure S4



Supplementary Figure Legends

Supplemental Figure S1. hsCRP induces ROS in HUVECs. HUVECs were plated on chamber slides and 18 hrs later cells were incubated with the Oxidative Stress Detection Reagent from the Total ROS detection kit (Enzo Life Sciences) in serum-free media for 30 min. Cells were washed with PBS and were either mock treated (Untrt) or treated for 10 min with either 25 μg/ml hsCRP or 100 μM H_2O_2. Fluorescent images (visualizing ROS) or phase pictures were taken on a Zeiss Observer D1 microscope with an AxioCam1Cc1 camera.

Supplemental Figure S2. Effects of hsCRP on the expression of NADPH oxidases and antioxidant enzymes. (A) HUVECs were incubated in a 1:10 dilution of growth media for 18 hrs in the absence (Untrt) or presence of hsCRP (25 μg/ml). Cells were lysed, separated by SDS-PAGE and immunoblotted with anti-Nox2 (Abcam), anti-Nox4 (Santa Cruz), anti-Nox5 (Abcam), anti-catalase (Calbiochem), anti-SOD1 (Calbiochem), anti-SOD2 (Abcam), anti-glutathione peroxidase (GPx; Abcam), and anti-actin (Santa Cruz) antibodies as a protein loading control. To examine SOD1 expression, we loaded only 1/6 of the sample volume (lower immunoblots). (B) Relative levels of the indicated proteins were quantified from immunoblots and normalized to actin control. The histogram represents the normalized mean from 4 independent experiments + SEM. *P<0.05 by Student's t-test.

Supplemental Figure S3. Effects of hsCRP on different forms of DNA damage. (A) HUVECs were mock treated or treated with 100 μM H_2O_2 or 25 μg/ml hsCRP for 30 or 60 min in serum-free media. The alkaline comet assay was performed as previously described [1, 2]. The comets were visualized using an Eclipse E-400 fluorescence microscope (Nikon, Japan) attached to a Pulnix video camera (Kinetic Imaging, LTD, Liverpool, UK), and were analyzed using Komet 5.5 software (Kinetic Imaging LTD). Olive tail moment was used as a measure of DNA damage level [3, 4]. Analogous results were obtained when % tail DNA was examined. The histogram represents the mean $\pm$ SEM from four experiments. **P<0.01 by one-way ANOVA and Tukey's post-hoc test. (B) HUVECs were treated with 25 μg/ml hsCRP for the indicated time points or with 2 μM staurosporine for 4 hrs. Cells were stained with anti-γ-H2AX antibodies (Millipore; Cat # 16-202A (clone JBW301)) or DAPI according to manufacturer's instructions.

Supplemental Figure S4. hsCRP increases MDA levels in HUVECs. HUVECs (2 15 cm dishes/condition) were untreated or treated with 25 μg/ml hsCRP for 30 min in SF media. Cells were washed in PBS, sonicated, centrifuged at 3000 x *g* for 10 min and the supernatant was used directly for MDA analysis using the BIOXYTECH® MDA-586 kit according to manufacturer's instructions (OxisResearch™). MDA concentration was calculated according to a standard curve and was normalized to the amount of protein in the sample. The histogram represents the normalized mean + SEM from 4 independent experiments. *$p<0.05$ using Student's t-test.

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