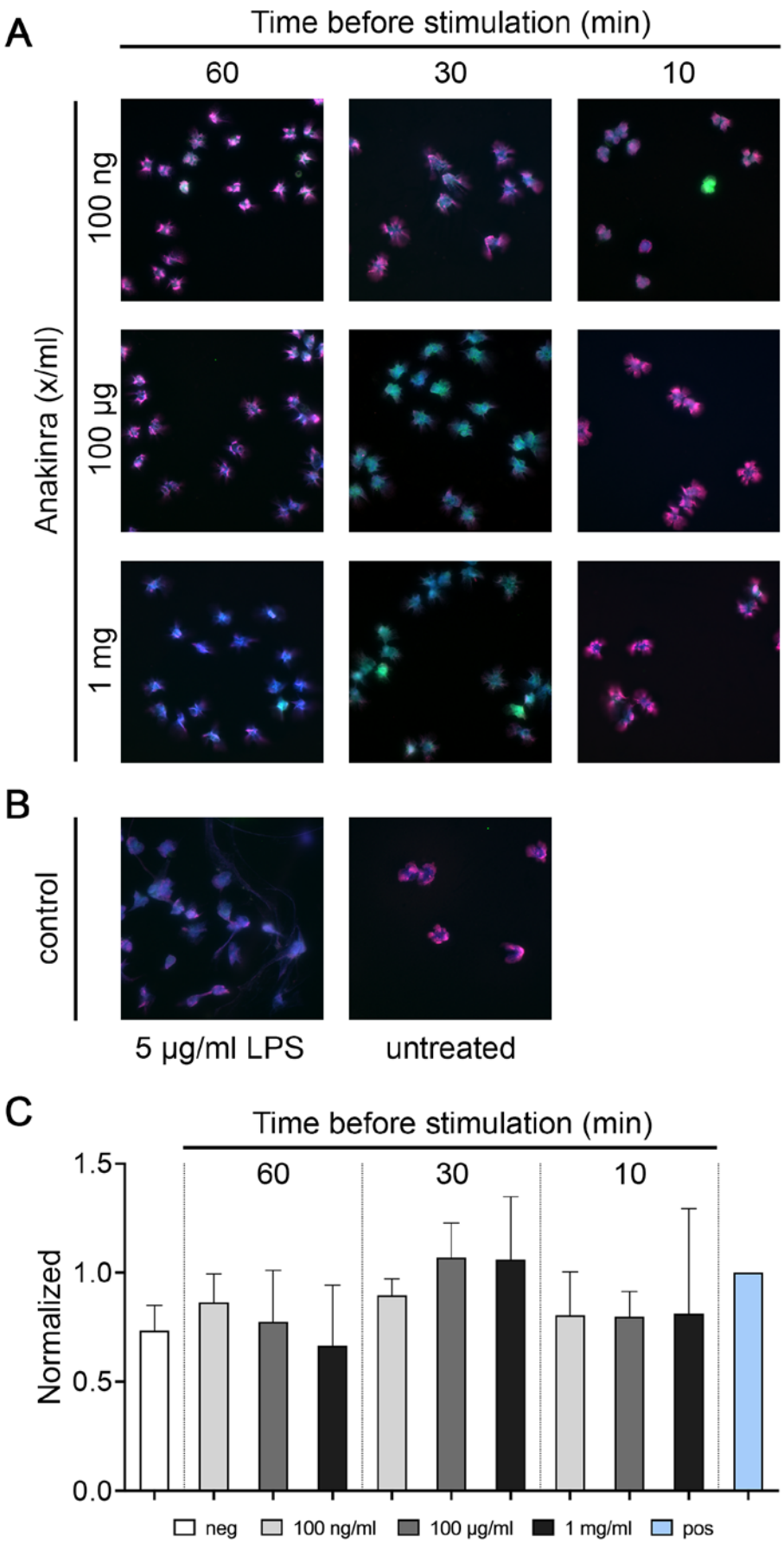
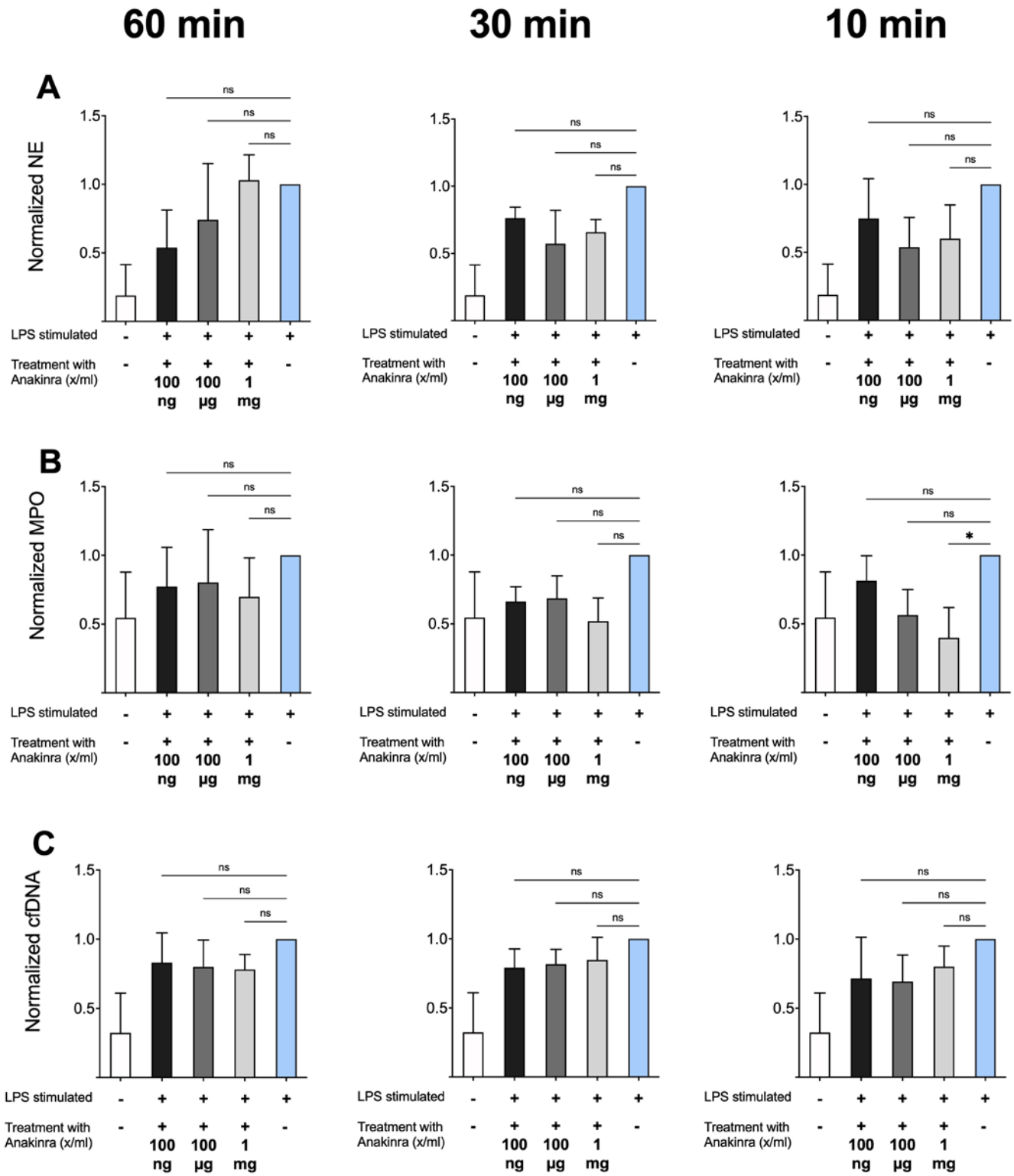
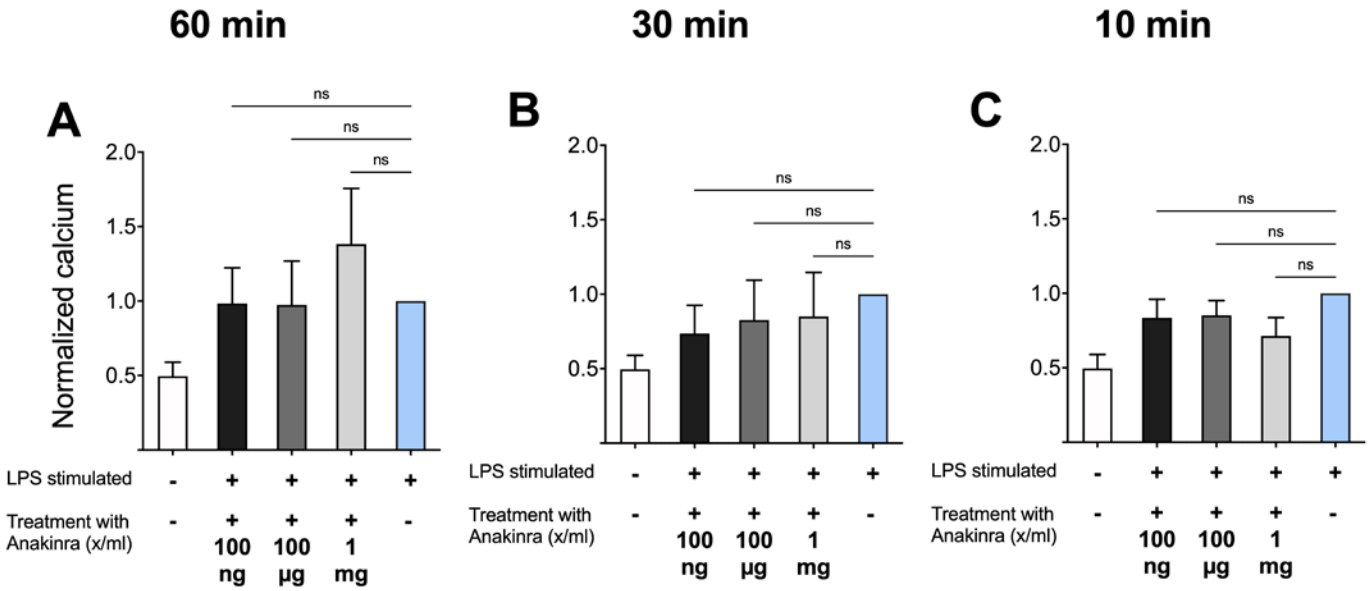
Supplementary



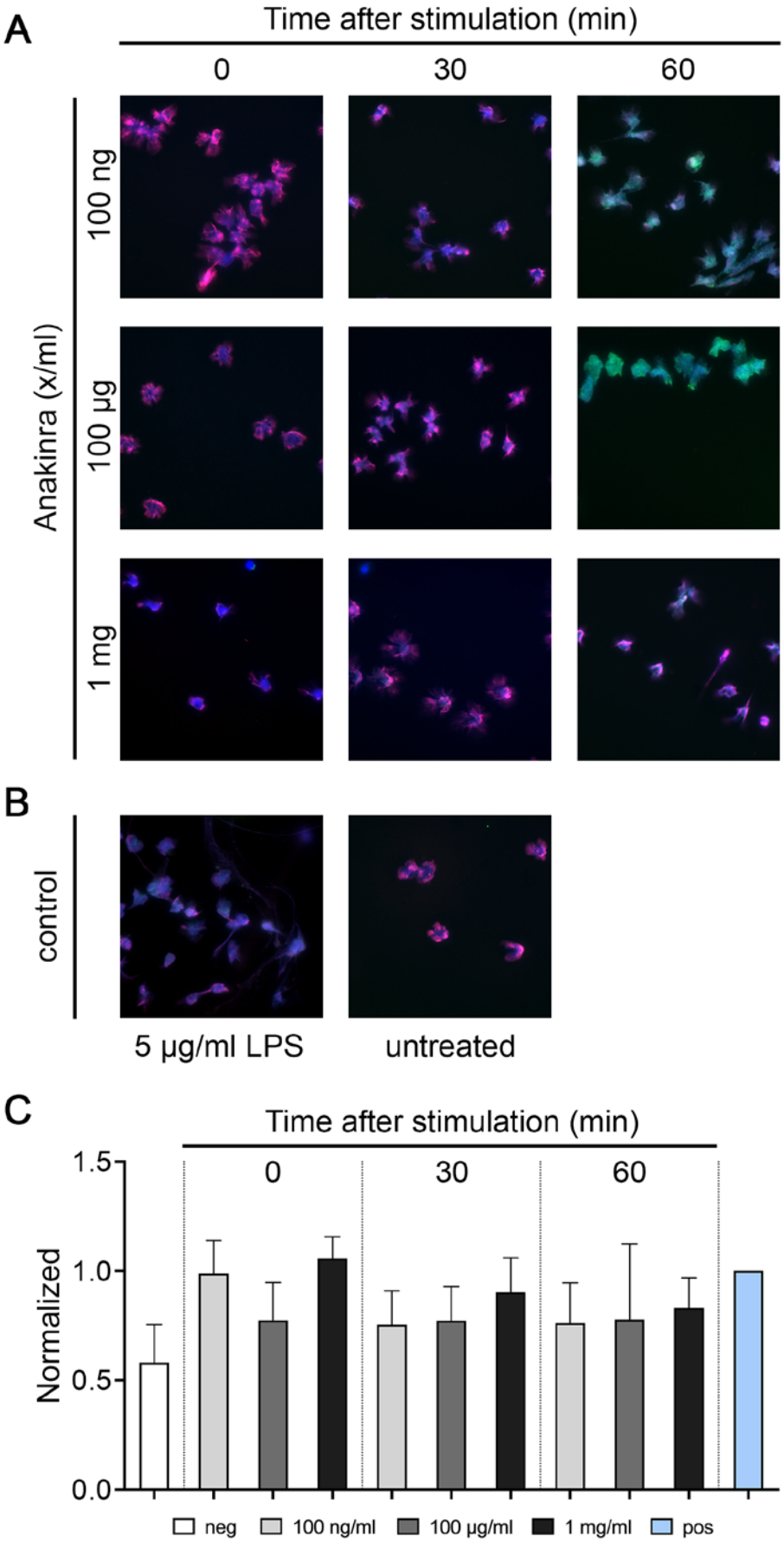
Supplementary Fig. 1. Immunofluorescence staining of anakinra pretreated and LPS-stimulated neutrophils. Cells were pretreated with anakinra (100 ng/ml, 100 µg/ml, 1 mg/ml) for 60, 30 and 10 minutes before stimulation with 5 µg/ml LPS (E.coli O55:B5) for 3 hours (**A**). DNA was stained with DAPI (blue), NE (pink/AF 647) and MPO (green/FitC). Positive control: Cells were not pretreated with anakinra and stimulated with 5 µg/ml LPS (E.coli O55:B5) for 3 hours negative control cells did not receive any treatment (**B**). Graphics show averages ± SD of mean gray value of signal per area of five images per well performed in triplicate and analyzed with ImageJ software (**C**). DNA and markers were stained as described before and imaged at a magnification of 40×



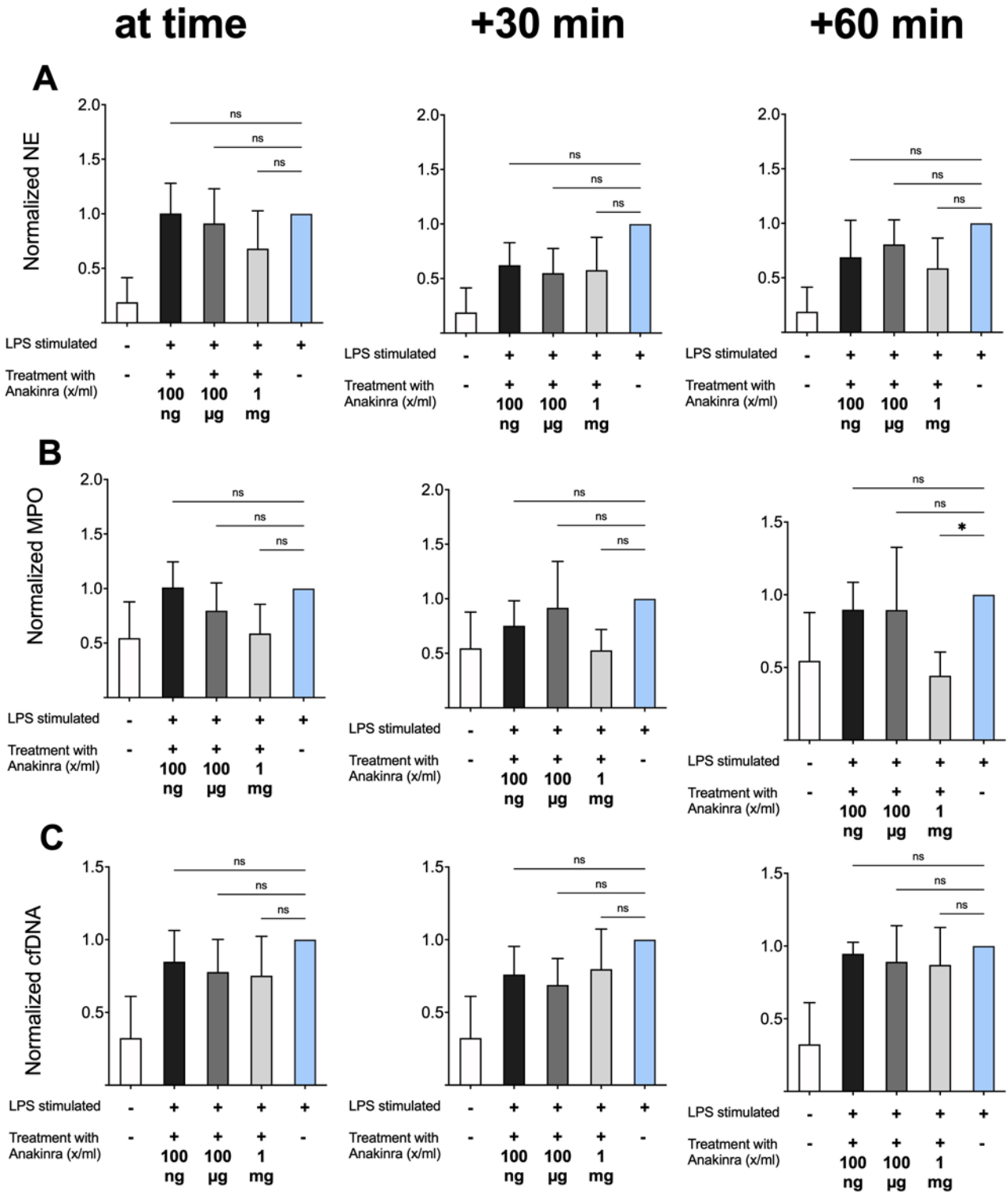
Supplementary Fig. 2. Changes of markers of NET formation in anakinra pretreated and LPS-stimulated neutrophils. Cells were pretreated with anakinra in concentrations of 100 ng/ml, 100 µg/ml and 1 mg/ml for 60, 30 and 10 minutes. Afterwards, cells were stimulated with LPS 5 µg/ml LPS (E.coli O55:B5) to induce NET formation. LPS stimulation was performed for a total of 3 hours to induce NET formation. Normalized data for NE activity are shown (**A**), normalized data for MPO activity are shown (**B**) and results for normalized cfDNA are shown (**C**). All data are values of absorbance/fluorescence normalized to LPS-stimulated controls



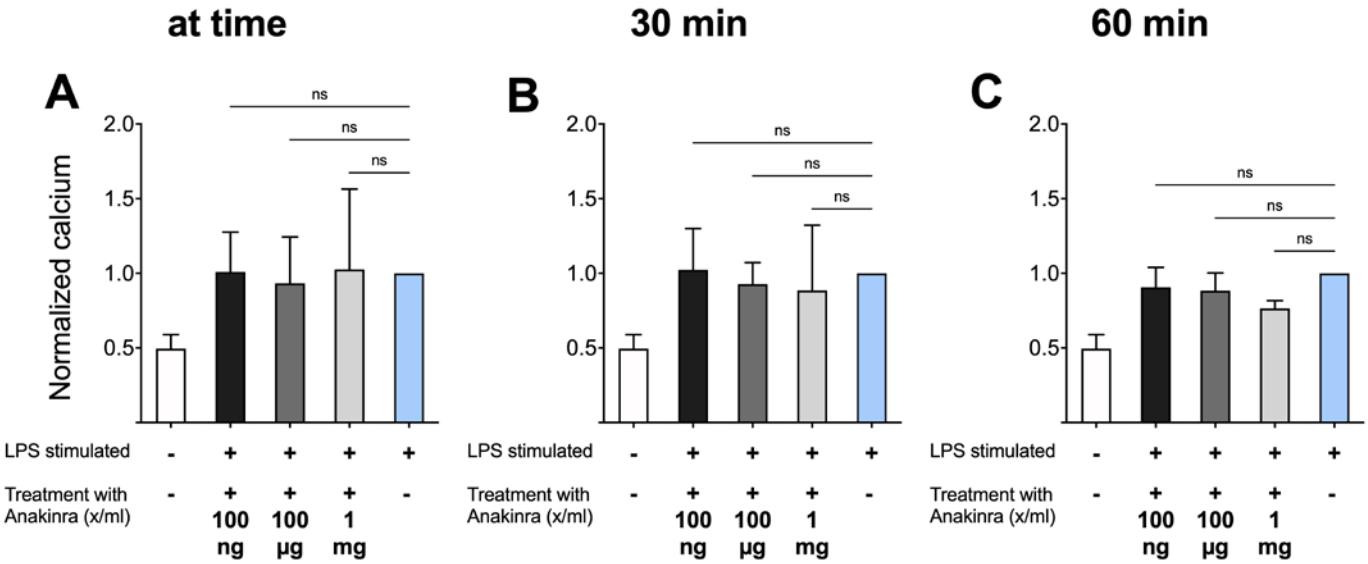
Supplementary Fig. 3. Calcium influx in anakinra pretreated and LPS-stimulated neutrophils. Cells were treated with anakinra in concentrations of 100 ng/ml, 100 µg/ml and 1 mg/ml for 60 (**A**), 30 (**B**) and 10 minutes (**C**). Afterwards, cells were stimulated with LPS 5 µg/ml (E.coli O55:B5) to induce NET formation. After stimulation with LPS calcium influx was measured. LPS stimulation was performed for a total of 3 hours. All data are normalized values of fluorescence to LPS-stimulated controls



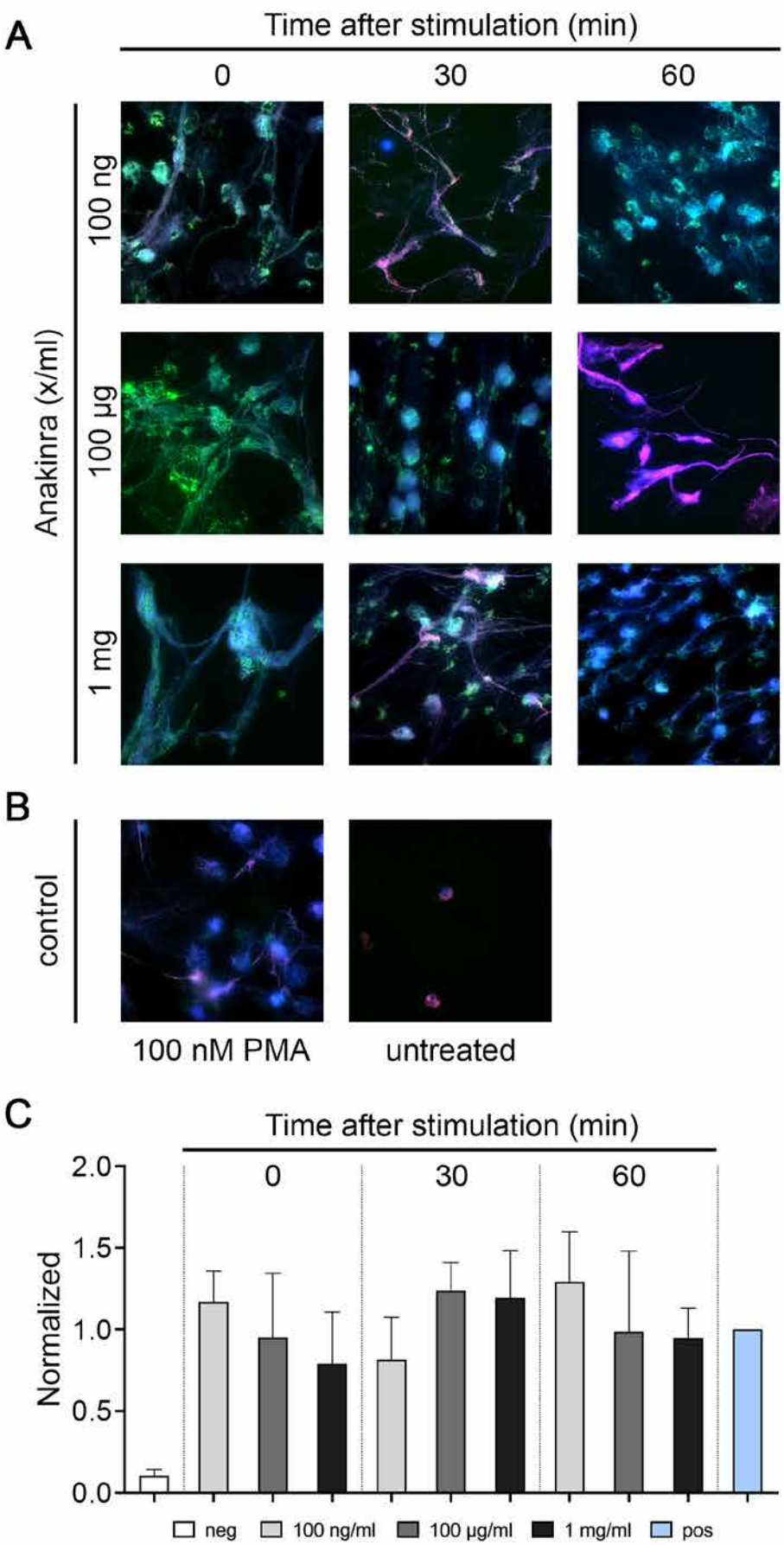
Supplementary Fig. 4. Immunofluorescence staining of anakinra treated and LPS-stimulated neutrophils. Cells were treated with anakinra in concentrations of 100 ng/ml, 100 µg/ml and 1 mg/ml at time, 30 minutes and 60 minutes after stimulation with 5 µg/ml LPS (E.coli O55:B5). LPS stimulation was performed for a total 3 hours to induce NET formation (**A**). DNA was stained with DAPI (blue), NE (pink/AF 647) and MPO (green/FitC). Positive control: Cells were not treated with anakinra and stimulated with 5 µg/ml LPS (E.coli O55:B5) for 3 hours (**B**). DNA and markers were stained as described before and imaged at a magnification of 40×. Graphics show averages ± SD of mean gray value of signal per area of five images per well performed in triplicate and analyzed with ImageJ software (**C**)



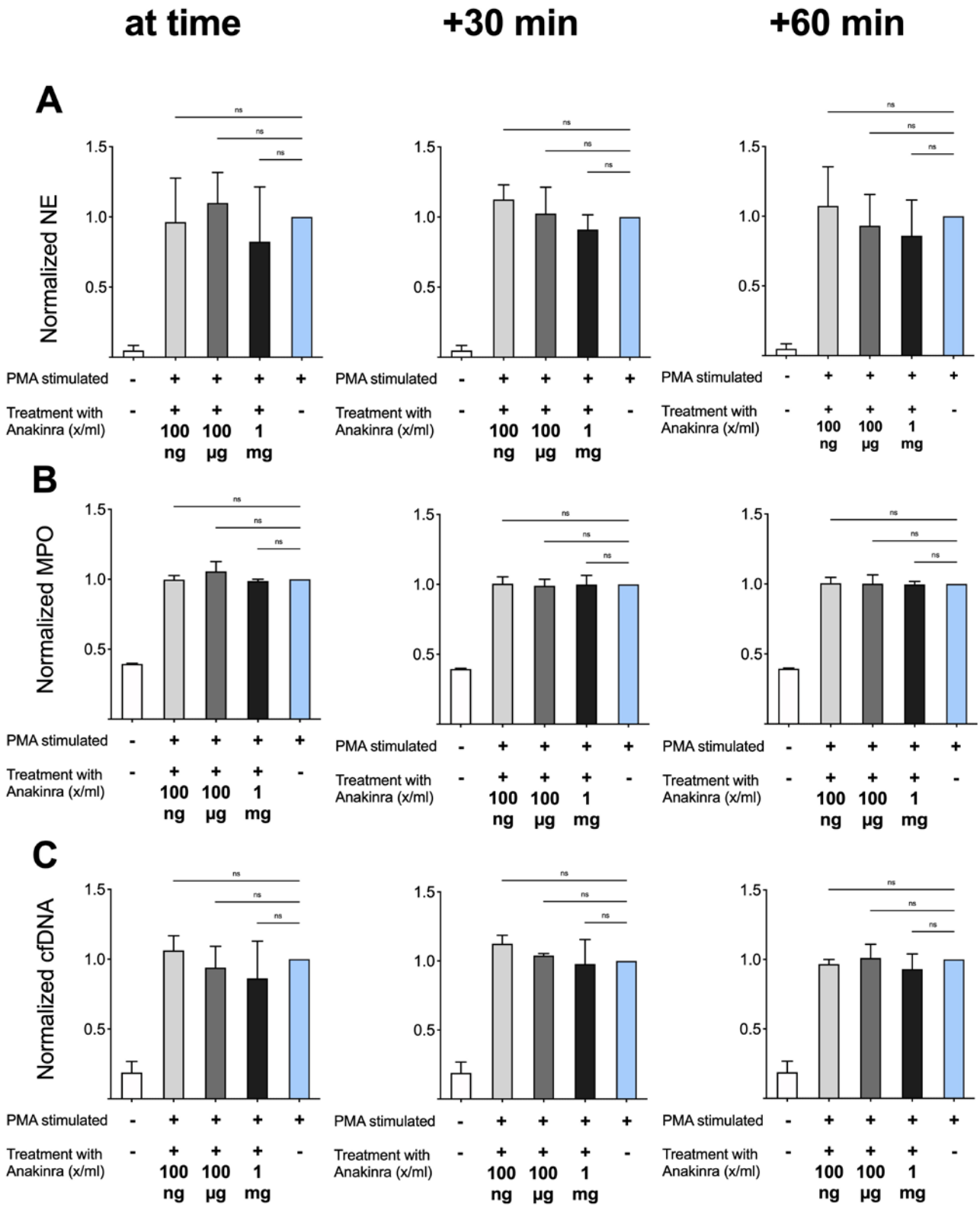
Supplementary Fig. 5. Changes of markers of NET formation in anakinra treated and LPS-stimulated neutrophils. Cells were treated with anakinra in concentrations of 100 ng/ml, 100 µg/ml and 1 mg/ml at time, 30 minutes and 60 minutes after stimulation with LPS 5 µg/ml LPS (E.coli O55:B5). LPS stimulation was performed for a total of 3 hours to induce NET formation. Normalized data for NE activity are shown in (**A**), normalized data for MPO activity are shown in (**B**) and results for normalized cfDNA are shown in (**C**). All data are values of absorbance/fluorescence normalized to LPS-stimulated controls



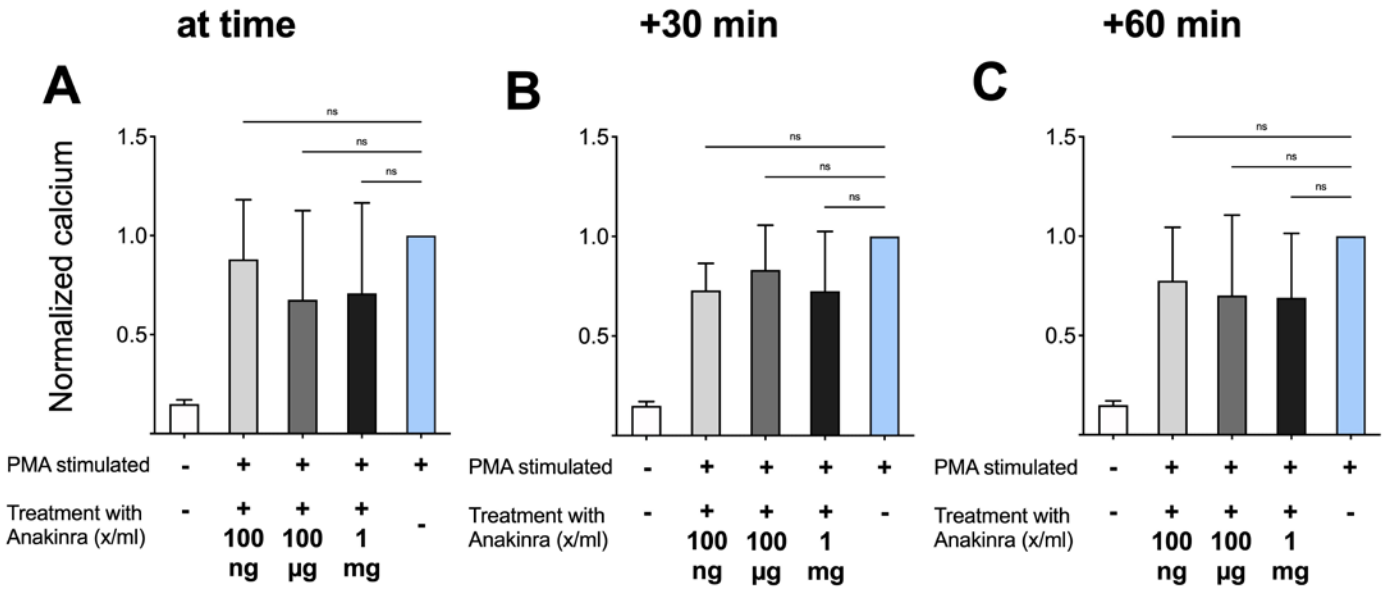
Supplementary Fig. 6. Calcium influx in anakinra treated and LPS-stimulated neutrophils. Cells were treated with anakinra in concentrations of 100 ng/ml, 100 µg/ml and 1 mg/ml at time (**A**), 30 minutes (**B**) and 60 minutes (**C**) after stimulation with 5 µg/ml LPS (E.coli O55:B5) and calcium influx was measured. LPS stimulation was performed for a total of 3 hours to induce NET formation. All data are values of fluorescence normalized to LPS-stimulated controls



Supplementary Fig. 7. Immunofluorescence staining of anakinra treated and PMA-stimulated neutrophils. Cells were treated with anakinra in concentrations of 100 ng/ml, 100 µg/ml and 1 mg/ml at time, 30 minutes and 60 minutes after stimulation with 100 nM PMA. PMA stimulation was performed for a total 3 hours to induce NET formation (**A**). DNA was stained with DAPI (blue), NE (pink/AF 647) and MPO (green/FitC). Positive control: Cells were not treated with anakinra and stimulated with 100 nM PMA for 3 hours (**B**). Graphics show averages ± SD of mean gray value of signal per area of five images per well performed in triplicate and analyzed with the ImageJ software (**C**). DNA and markers were stained as described before and imaged at a magnification of 40×



Supplementary Fig. 8. Changes of markers of NET formation in anakinra treated and PMA-stimulated neutrophils. Cells were treated with anakinra in concentrations of 100 ng/ml, 100 µg/ml and 1 mg/ml at time, 30 minutes and 60 minutes after stimulation with 100 nM PMA. PMA stimulation was performed for a total of 3 hours to induce NET formation. Normalized data for NE activity are shown in (**A**), normalized data for MPO activity are shown in (**B**) and results for normalized cfDNA are shown in (**C**). All data are values of absorbance/fluorescence normalized to PMA-stimulated controls



Supplementary Fig. 9. Calcium influx in anakinra treated and PMA-stimulated neutrophils. Cells were treated with anakinra in concentrations of 100 ng/ml, 100 µg/ml and 1 mg/ml at the time of (**A**), 30 minutes (**B**) and 60 minutes (**C**) after stimulation with 100 nM PMA and calcium influx was measured. PMA stimulation was performed for a total of 3 hours to induce NET formation. All data are values of fluorescence normalized to PMA-stimulated controls