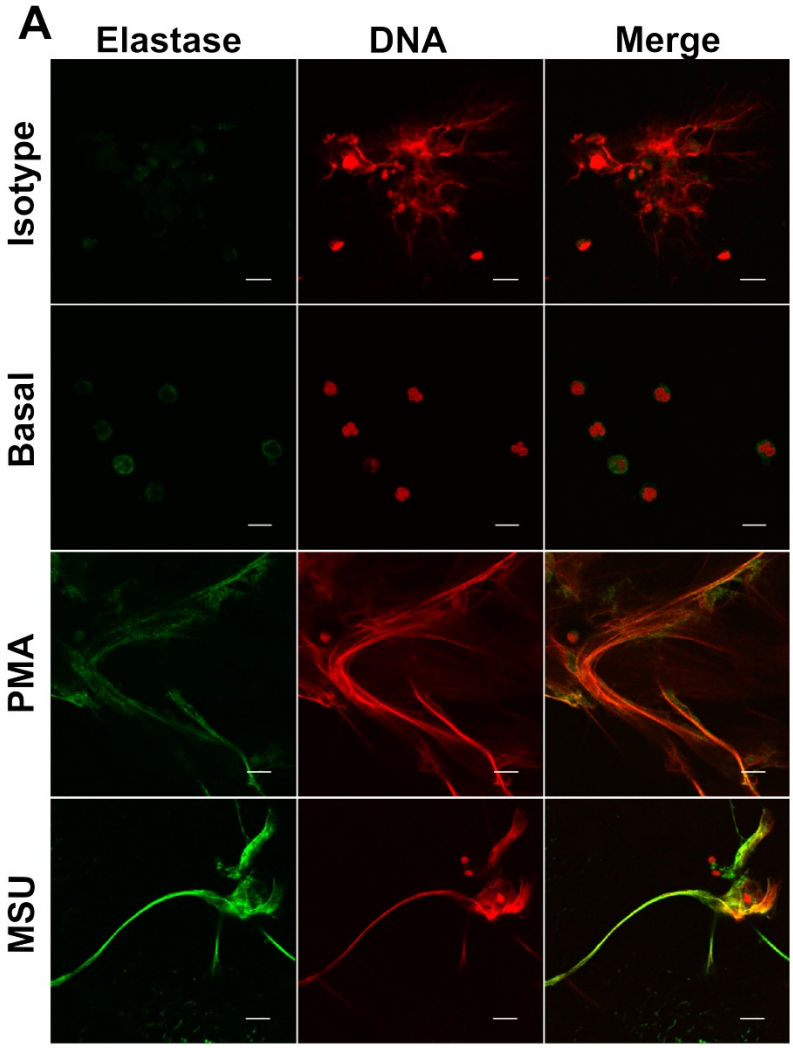
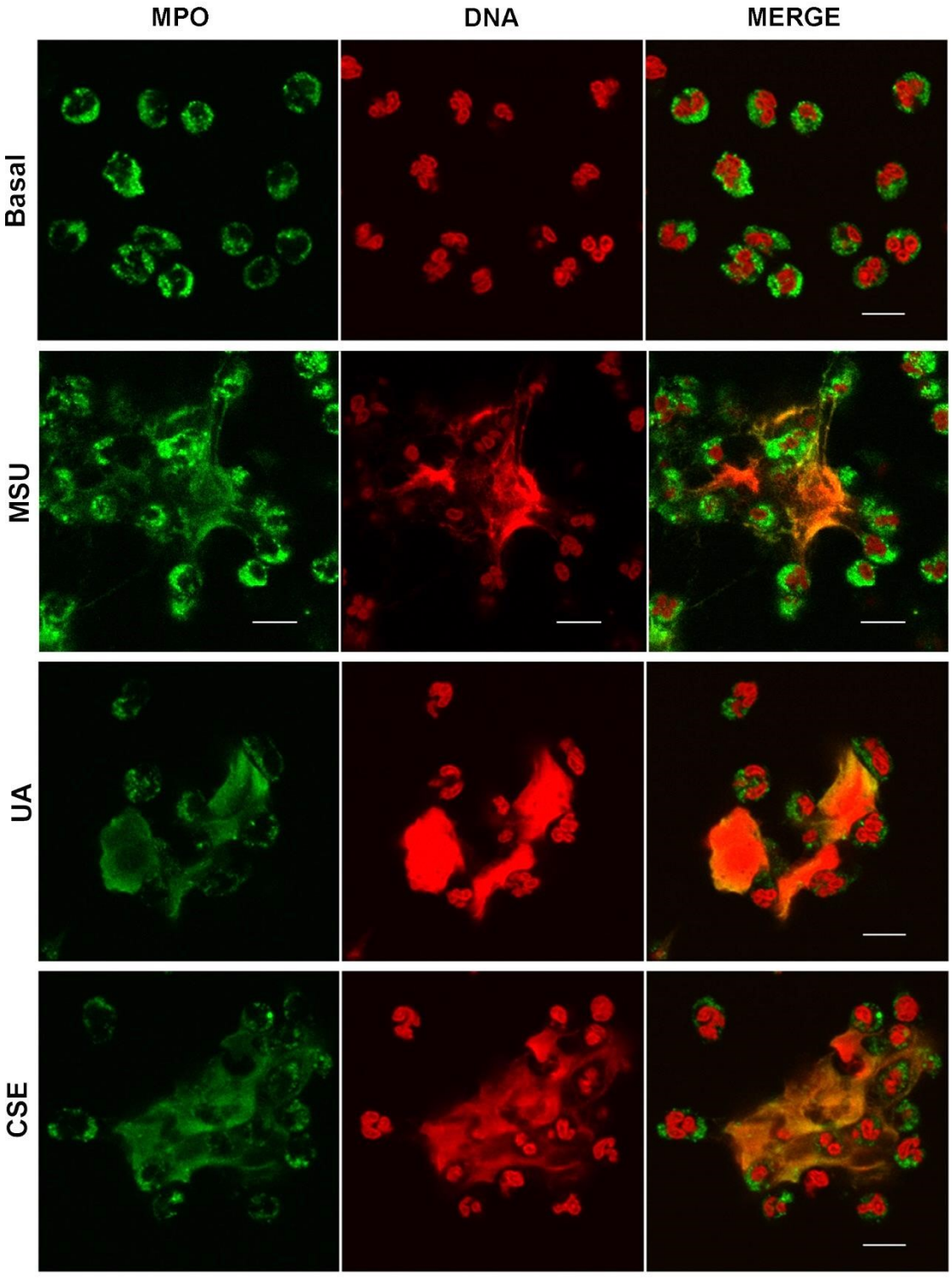
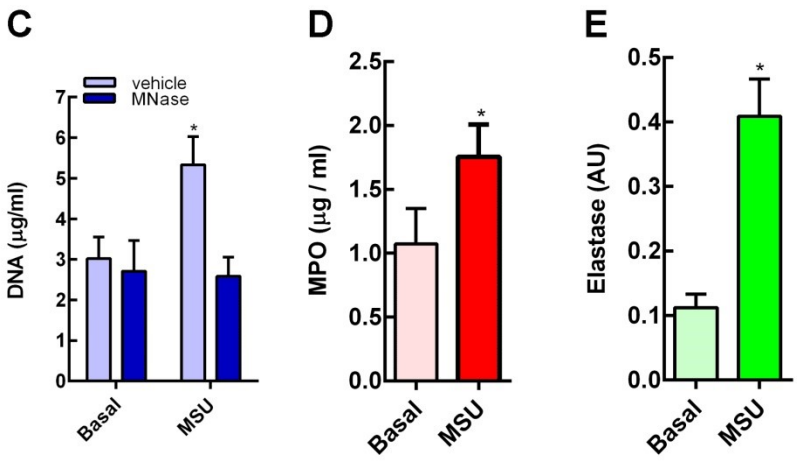
Figure S5. MSU crystals, uric acid (UA) or cigarette smoke extract (CSE) stimulated NET release.



B





Neutrophils were stimulated with either phorbol myristate acetate (PMA; 50 ng/ml) as positive control, MSU crystals (300 µg/ml), uric acid (UA; 8 mg/dl), cigarette smoke extract (CSE; tenfold dilution of a solution with a concentration corresponding to cigarettes containing 1.36 mg/ml tar) or left untreated (basal) for 4 h at 37°C. Then, samples were fixed with 4% PFA, permeabilized with 0.5% Triton X-100, and then DNA was stained with PI (red), and elastase (A) and myeloperoxidase (MPO; B) were stained with specific antibodies (green). Images were acquired by using a FluoView FV1000 confocal microscope. Images are representative of three independent experiments. Scale bar: 10 µm. (C) DNA concentrations in supernatants of neutrophils stimulated for 4 h with MSU, after separation from cell debris in the presence or absence of microccocal nuclease (MNase; 10 U/ml). (D) MPO concentration and (E) elastase activity represented by arbitrary units of fluorescence (AU), in supernatants of neutrophils stimulated for 4 h with MSU crystals. Data are depicted as the mean ± SEM of 4 (B), 3 (C) and 4 (D) independent experiments. One way ANOVA (B) and Student´s t test (C and D); *p<0.05 compared to basal.