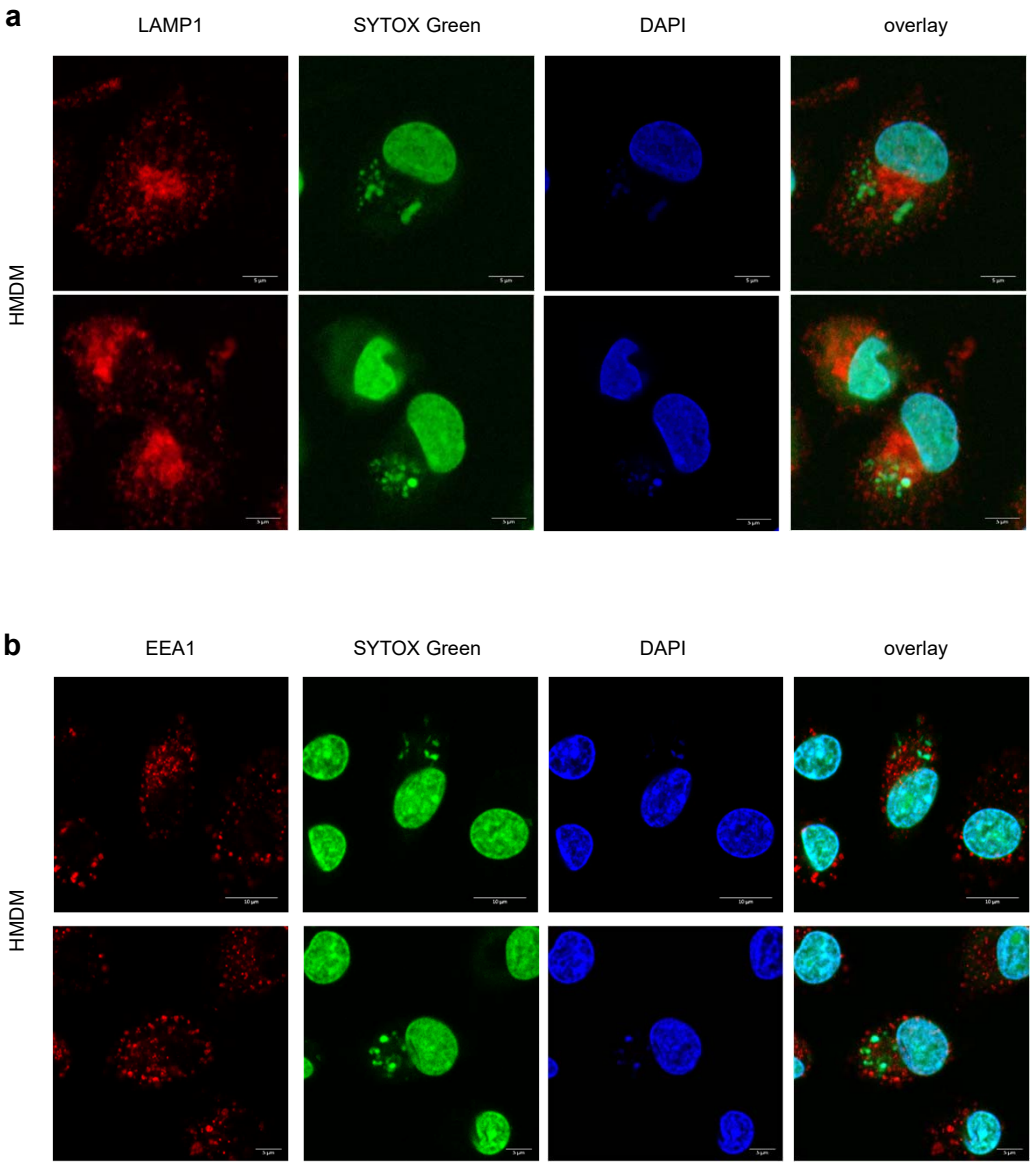
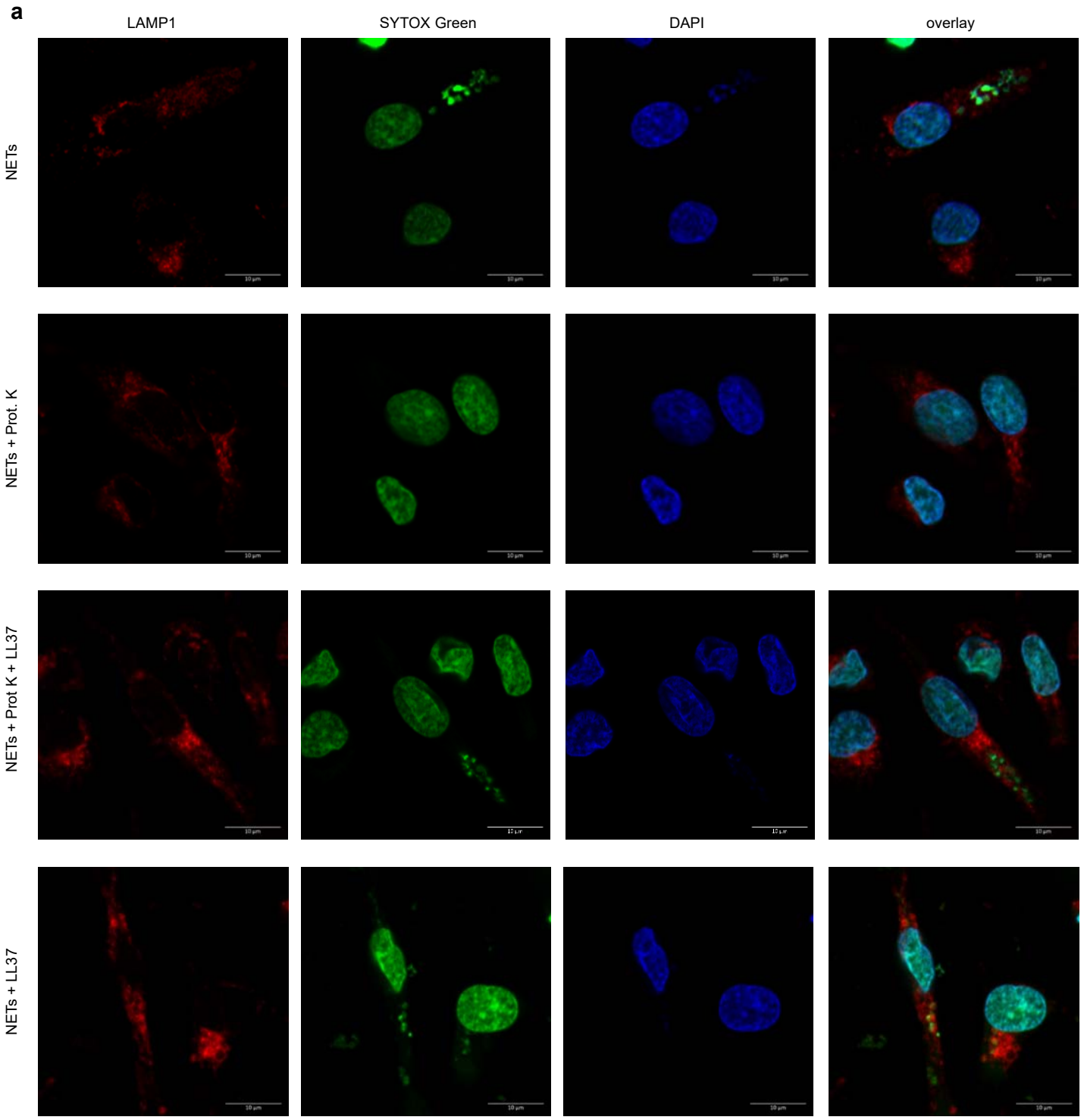
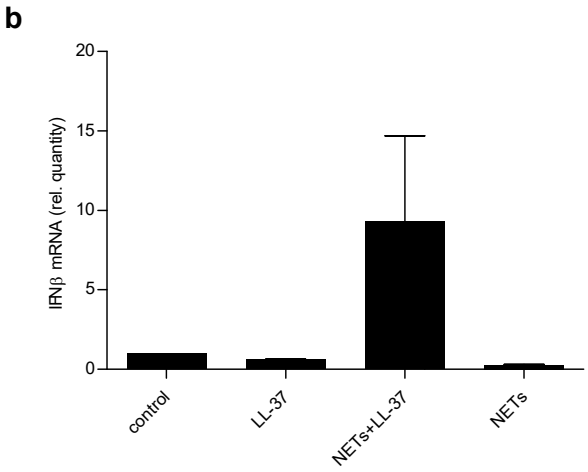
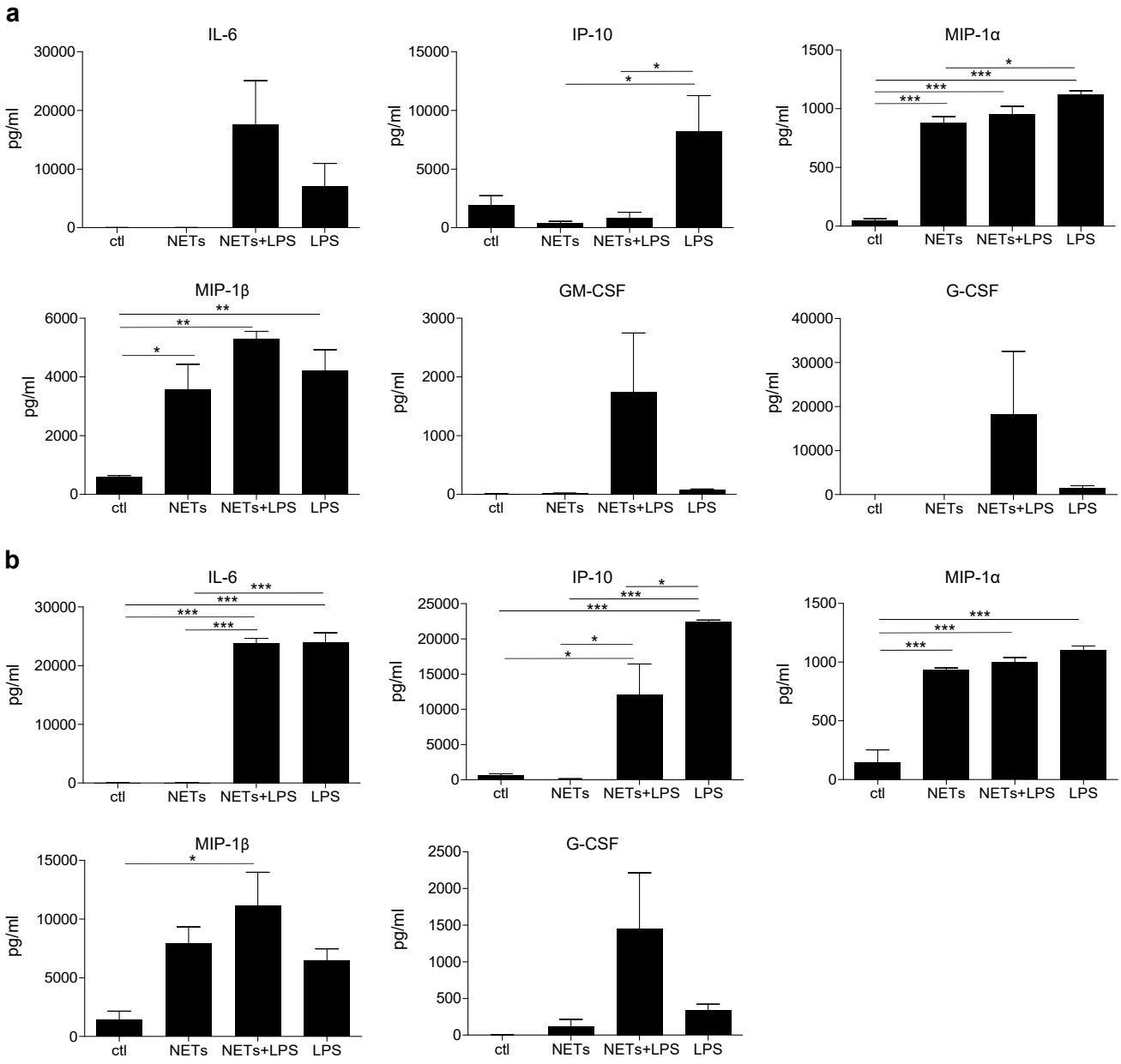


Suppl. Figure S1. NETs do not co-localize with endosomes or lysosomes. HMDMs were incubated for 1 h with SYTOX® green pre-stained NETs (15 min). After fixation, immunostaining for (a) lysosomal compartments with LAMP-1 (red) or (b) early endosomal compartments with EEA1 (red) was carried out and representative confocal images were taken. Scale bars are 5 µm (a and b, lower panels) and 10 µm (b, upper panels).

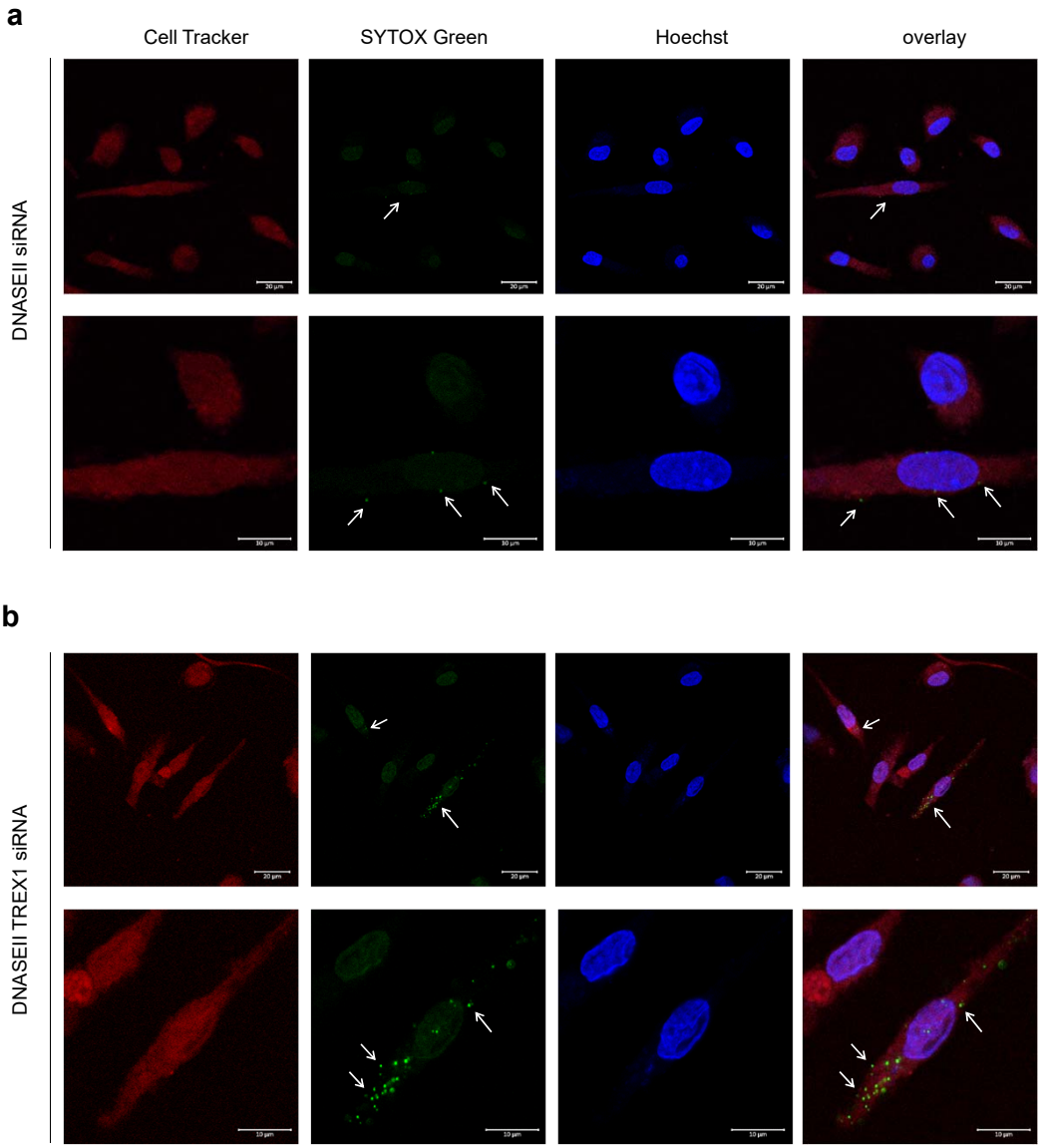




Suppl. Figure S2. NETs complexed with LL-37 do not co-localize with lysosomes. (a) Representative confocal images of HMDMs co-incubated for 1 h with NETs or NETs subjected to Proteinase K (200 µg/ml) treatment with or without addition of LL-37 (10 µM) and labeled with SYTOX® green (15 min). After fixation, cells were stained for LAMP1 (red) and counterstained with DAPI to visualize nuclei (blue). Scale bars are 10 µm. (b) HMDMs were incubated for 3 h with control medium, LL-37 (10 µM), or purified NETs with/without LL-37 (30 min pre-incubation). Cells were then collected and IFN-β mRNA analysis was performed. The data were normalized on the endogenous level of *GAPDH* mRNA.



Suppl. Figure S3. Cytokine profiling. Profiling of soluble mediators released by (a) HMDMs or (b) MDDCs exposed to control medium, NETs, LPS (100 ng/mL), or NETs + LPS for 1 h and then to the relevant culture medium for 24 h, after which supernatants were collected and analyzed using the Luminex® platform. Data are shown as mean values ± S.E.M. of three independent experiments with three different blood donors. One-way ANOVA with Tukey post-test was used to compare all the groups and evaluate statistical significance expressed as follows: *p<0.05, **p<0.01, ***p<0.001; where not specified, differences between groups were found to be not significant. Note that not all the cytokines included in the multiplex array are shown here due to space restrictions; however, all results were included in the hierarchical cluster analysis (Figure 5).



Suppl. Figure S4. Role of intracellular nucleases for macrophage degradation of NETs. Representative confocal images of HMDMs transfected with specific siRNA targeting *DNASEII* (a) or a pool of siRNAs targeting *DNASEII* and *TREX1* (b) and co-incubated with purified NETs. Cells were stained with CellTracker Orange (red) and counterstained with Hoechst 33342 (blue) to visualize cell nuclei. Purified NETs were pre-stained with SYTOX® green and incubated with HMDMs for 1 h. Internalized NETs are evidenced as extranuclear SYTOX® green-positive DNA dots (lower panels, arrows) and cells presenting such dots (*i.e.*, undigested NETs) are indicated in the upper panels (arrows). Scale bar: 20 µm for the upper panel and 10 µm for the lower panels.