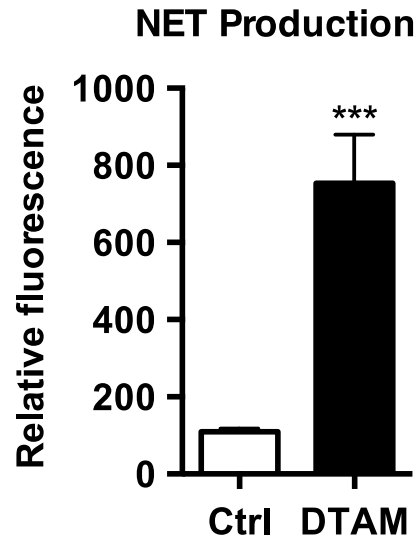
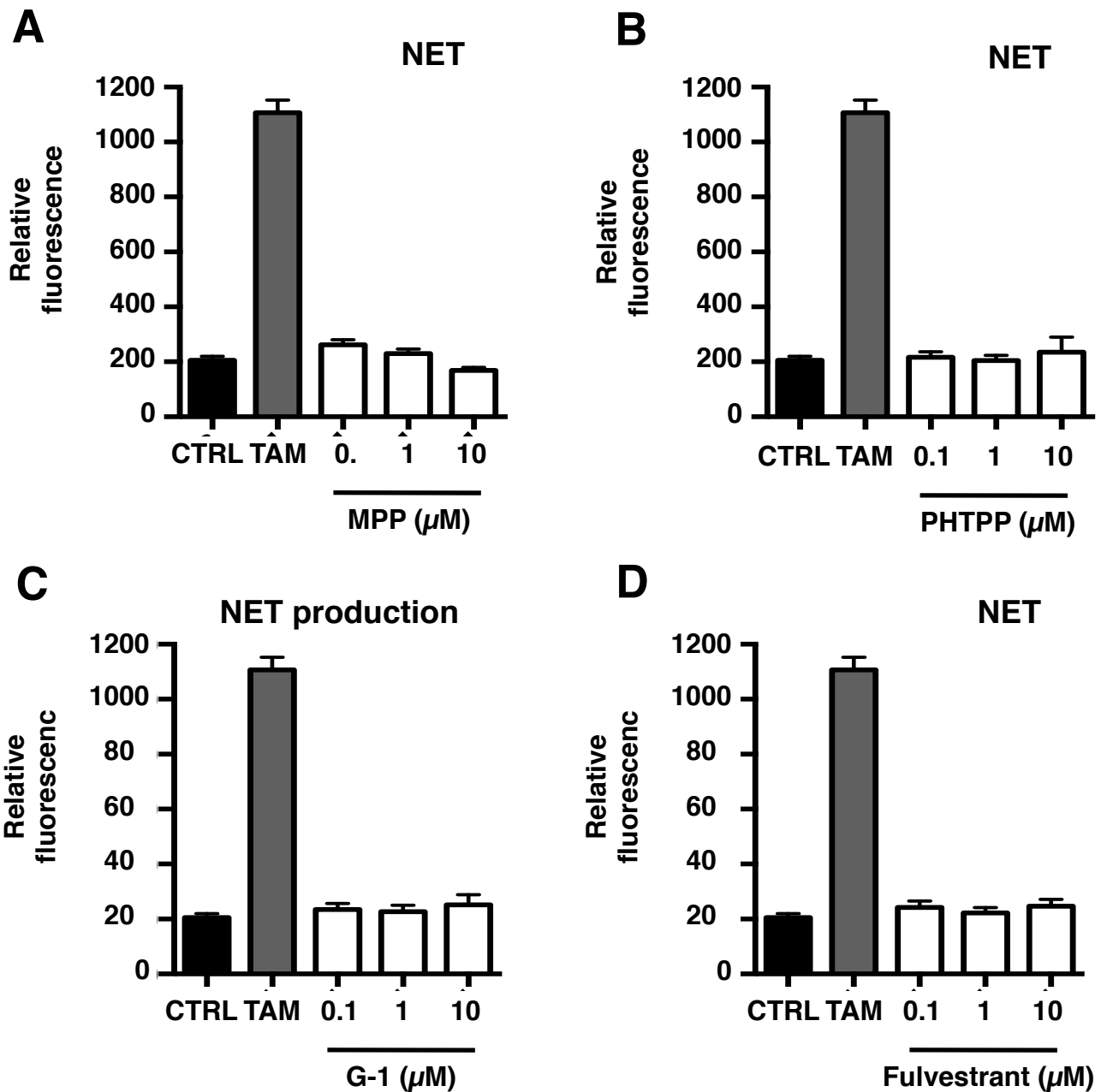


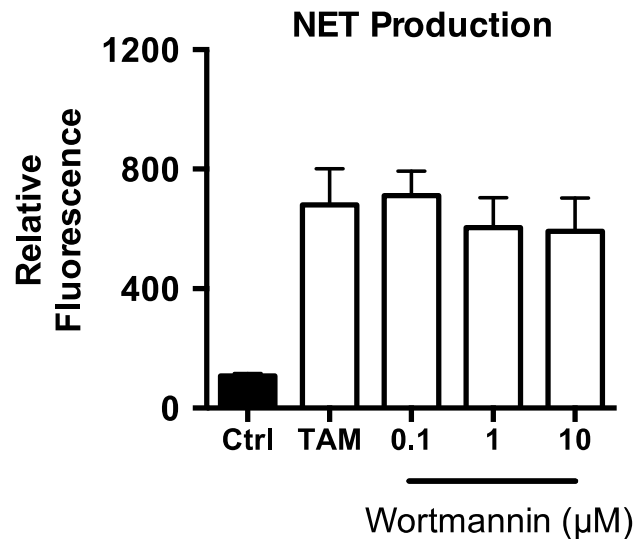
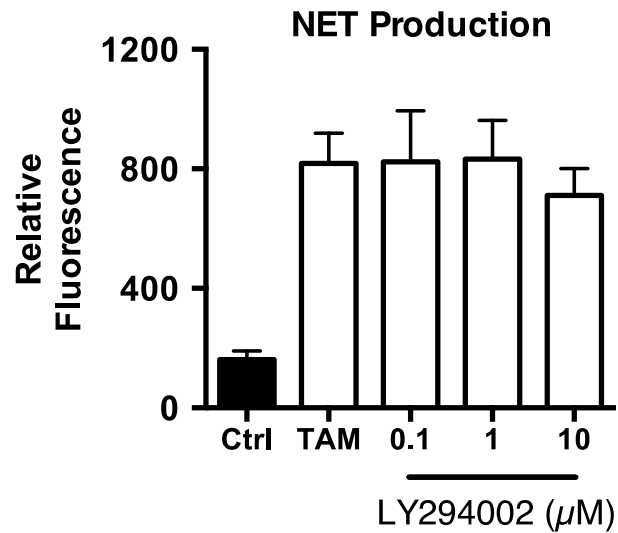
Supplementary Figure 1. Tamoxifen does not promote chemotaxis or chemokinesis in the absence of additional stimulation. Transwell chemotaxis assays revealed that tamoxifen treatment alone did not stimulate migration through a porous filter. N-Formyl-Met-Leu-Phe (fMLP), a potent stimulator of chemotaxis, was included as a positive control (n=9).



Supplementary Figure 2. N-Desmethyltamoxifen (DTAM) stimulates NET production. Human neutrophils were treated with DTAM (10 μ M) for 2 h. Quantification of extracellular DNA revealed significant NET production in response to DTAM treatment (n = 9). Results were analyzed by Student's t-test (***) $P < 0.001$ vs. control value).

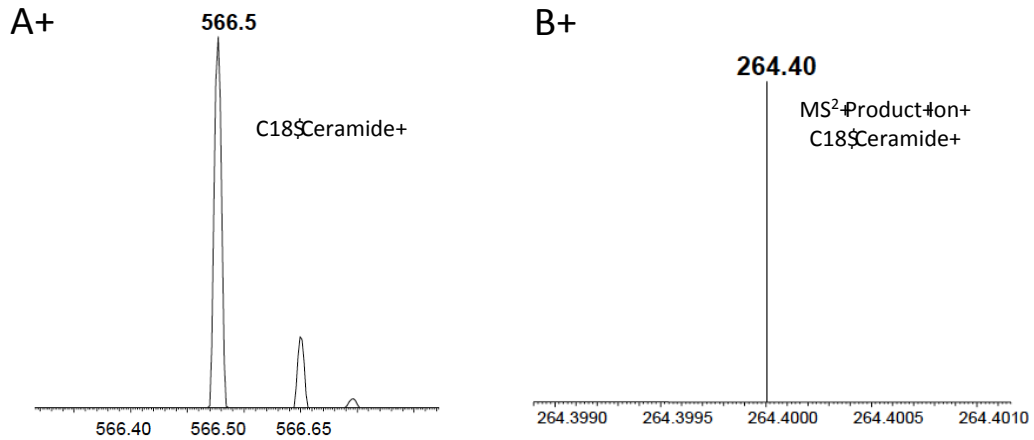


Supplementary Figure 3. Selective estrogen receptor agonists/antagonists do not stimulate NET production. Human neutrophils were treated with MPP (an ER α antagonist), PHTPP (an ER β antagonist), G-1 (a GPR30 agonist) or fulvestrant (a non-selective SERM that mimics the estrogen receptor effects of tamoxifen) at the indicated concentrations alongside 10 μ M tamoxifen. (n = 9 for each condition; p < 0.05 was taken to represent significance).

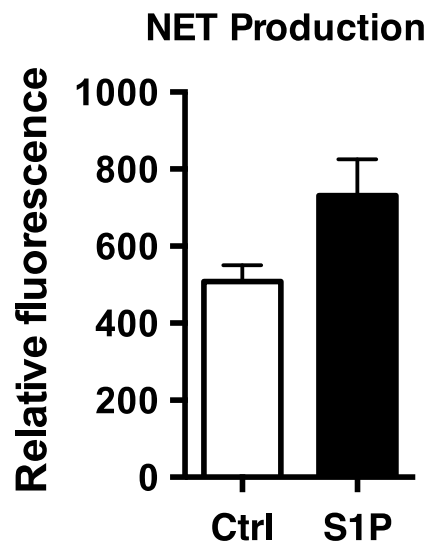


Supplementary Figure 4. PI3K inhibitors do not affect tamoxifen-induced NET production. Human neutrophils were preincubated with the PI3K inhibitors LY294002 or wortmannin for 1 hr at 37°C with 5% CO₂ prior to addition of 10 μM tamoxifen. NET production was assessed after a further 2 hr incubation under the same conditions (n = 9 for each condition; p < 0.05 was taken to represent significance). Neither LY294003 or wortmannin significantly inhibited tamoxifen-

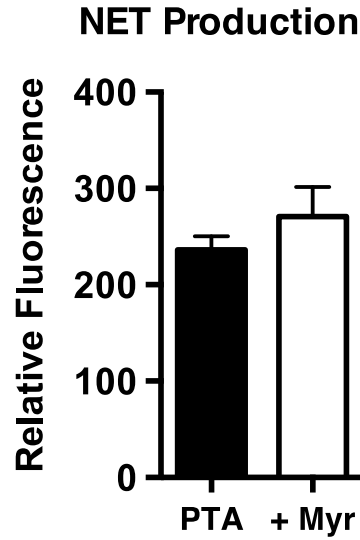
induced NET production. Results were analyzed by one-way ANOVA and post hoc Newman Keuls test.



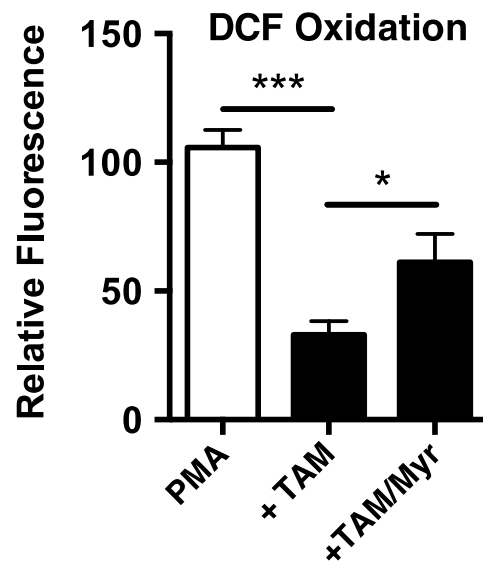
Supplementary Figure 5. Confirmation of presence of ceramide species by MS² ion verification. (A) Ceramide species were identified by the detection of precursor mono-isotopic masses consistent with previously reported values. (B) The presence of each ceramide was confirmed by the detection of a peak within an acceptable error range of the signature ceramide MS² product ion (m/z 264.3) after collision-induced dissociation (CID) of the ceramide parent masses.



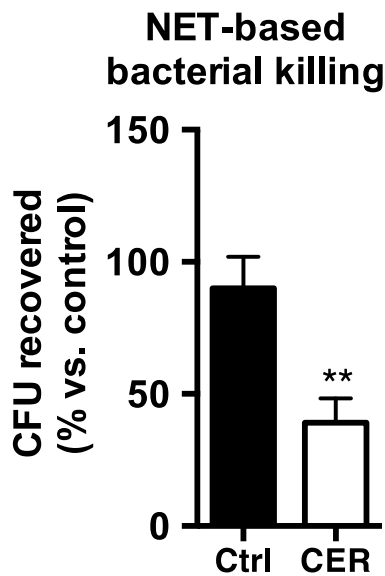
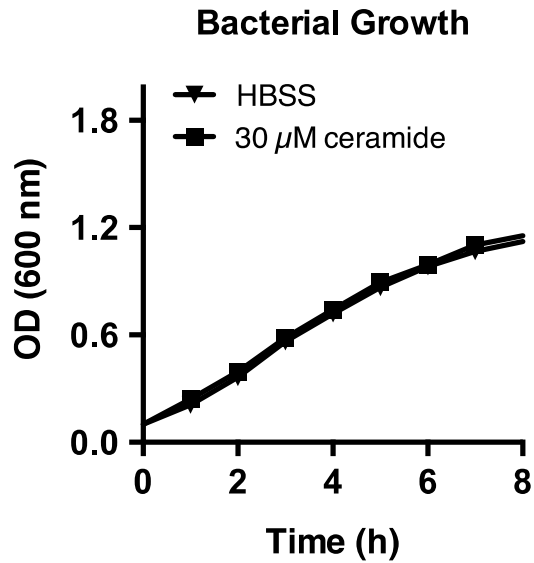
Supplementary Figure 6. Sphingosine-1-phosphate (S1P) does not significantly induce NET production. Human neutrophils were treated with S1P, which is generated from ceramide *in vivo*, for 2 hr at 37°C. Significant NET production was not observed (n = 9; p < 0.05 was taken to represent significance). Results were analyzed by Student's t-test.



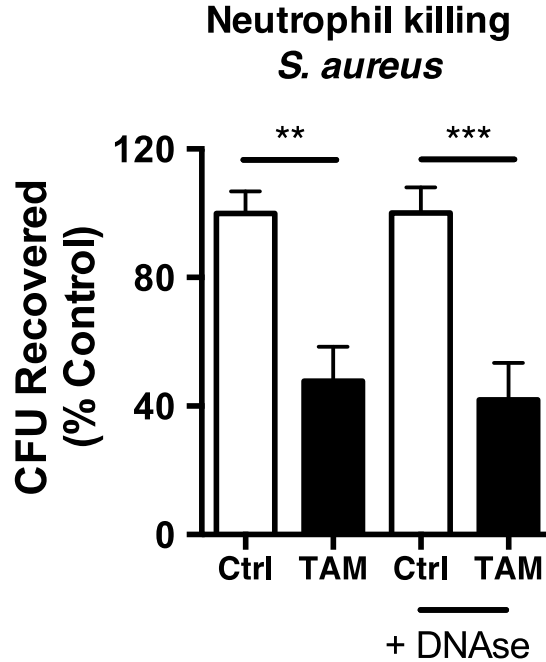
Supplementary Figure 7. Myriocin does not inhibit NET production in response to the PKC ζ agonist phosphatidic acid (PTA). To determine whether myriocin directly inhibits PKC ζ activity, neutrophils were pre-incubated for 90 min with 3 μ M myriocin. Cells were subsequently incubated with 10 μ M PTA and incubated for a further 3 hr, after which NET production was determined using PicoGreen as described in Methods (n = 9; p < 0.05 was taken to represent significance). No significant difference in NET production was observed in the myriocin-treated cells. Results were analyzed by Student's t-test.



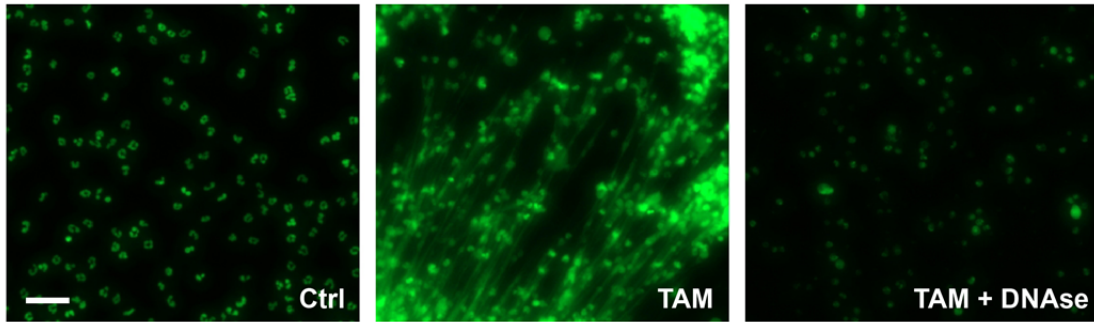
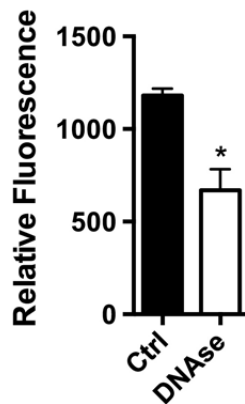
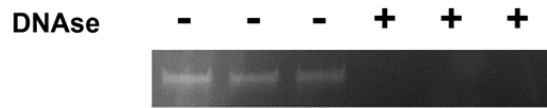
Supplementary Figure 8. Myriocin pre-treatment partially restores ROS production in tamoxifen-treated human neutrophils. After incubation with the fluorescent ROS probe DCF-AM (as described in Methods), human neutrophils were incubated for 90 min at 37C with 5% CO₂ in the presence or absence of 10 μM myriocin. PMA alone (25 nM), or PMA (25 nM) plus tamoxifen (10 μM) were added to wells. A significant reduction in PMA-induced ROS production was observed in the tamoxifen-treated cells at 15 min. This inhibition was partially and significantly reversed in myriocin-treated neutrophils. Results were analyzed by Student's t-test (* $P < 0.05$, *** $P < 0.001$ vs. control values).



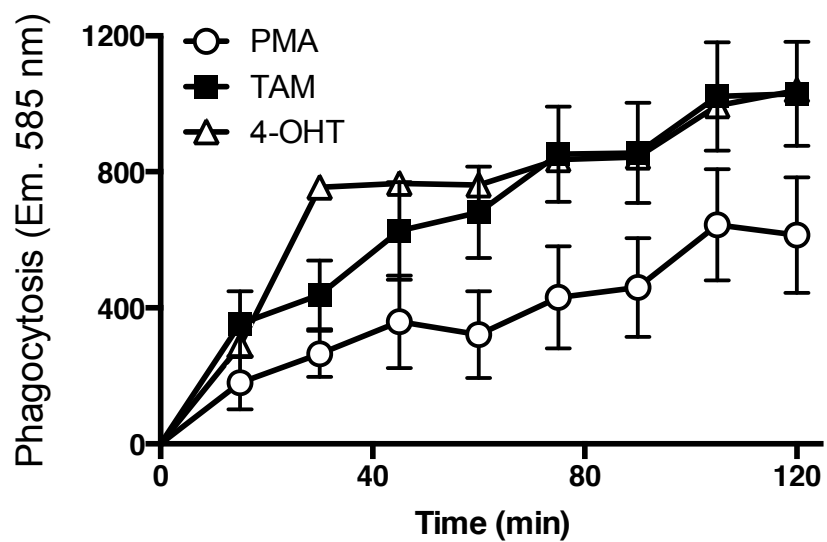
Supplementary Figure 9. Ceramide does not effect growth of bacteria but enhances NET-induced killing. Like Tamoxifen and 4-hydroxytamoxifen, ceramide (30 μM) had no effect on the growth of *Pseudomonas aeruginosa* (n = 6); a significant reduction in bacterial survival was observed in a NET-based bacterial killing assay in which neutrophils were pre-treated with 30 μM ceramide for 4 hours to stimulate NET production prior to addition of bacteria (n = 7-8). Results were analyzed by Student's t-test (** $P < 0.01$).



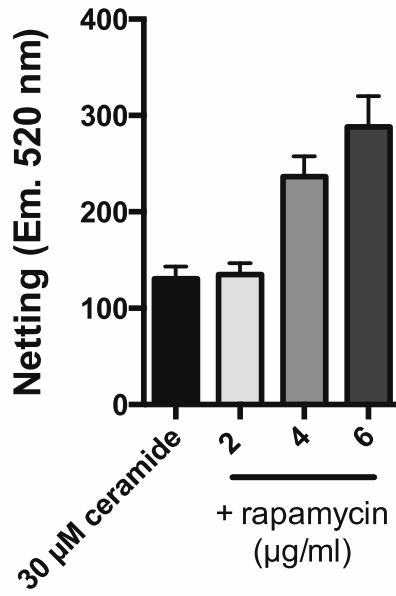
Supplementary Figure 10. Reductions in bacterial CFUs in NET-based killing assays are due to bacterial killing rather than clumping. NET-based killing assays were performed with a USA300 strain of methicillin-resistant *Staphylococcus aureus* as described in Methods; at the initial serial dilution step, samples from each well were paced in PBS containing 25 U/mL DNase for 30 minutes at RT prior to further dilution and plating. A statistically significant reduction in CFUs was observed in both untreated and DNase-treated samples, with no significant difference observed between the percent CFUs recovered from the tamoxifen-treated groups. Results were analyzed by Student's t-test (** $P < 0.01$, *** $P < 0.001$).

A**B****C**

Supplementary Figure 11. Assessment of stock DNase activity. **(A)** Neutrophils were incubated for 15 min with DNase at 37°C following a 105 min incubation with or without 10 μ M tamoxifen (followed by a single wash with PBS, fixing with paraformaldehyde, staining with SYTOX Green and imaging as described in Methods) to determine whether DNase treatment degraded NETs (scale bar = 50 μ m). **(B)** Lambda DNA (2.5 μ g) was incubated with DNase (the same aliquots used for the killing assays) for 30 min at room temperature; intact lambda DNA was quantified via the PicoGreen method and by running on a 1% agarose gel with ethidium bromide **(C)**. DNase used here was from the same preparation and at the same concentration (25 U/mL) used in killing experiments. Results were analyzed by Student's t-test (* $P < 0.05$).



Supplementary Figure 12. Tamoxifen and 4-hydroxytamoxifen enhance neutrophil phagocytosis. The effects of tamoxifen (TAM) and 4-hydroxytamoxifen (4-OHT; both 10 μ M) on neutrophil phagocytosis were assessed using pH-sensitive, fluorescent *Staphylococcus aureus*-labeled bioparticles (n = 10-12) as described in the Methods.



Supplementary Figure 13. Rapamycin enhances ceramide-induced NET production. Cells were pre-incubated with the autophagy-inducer rapamycin prior to stimulation with ceramide to determine its impact on NET production (n = 9).