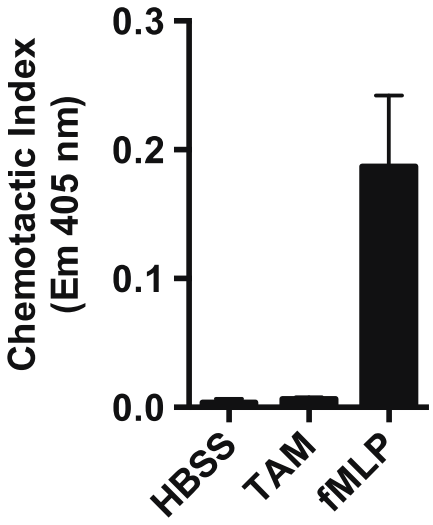
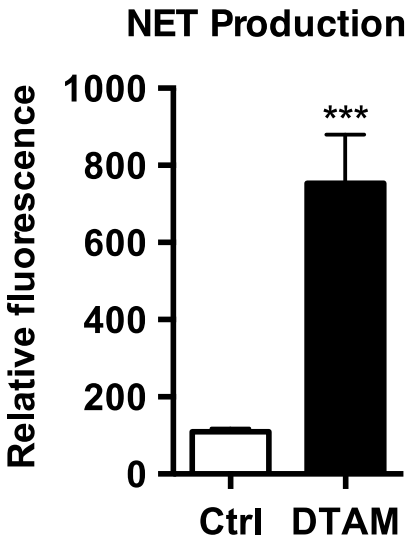
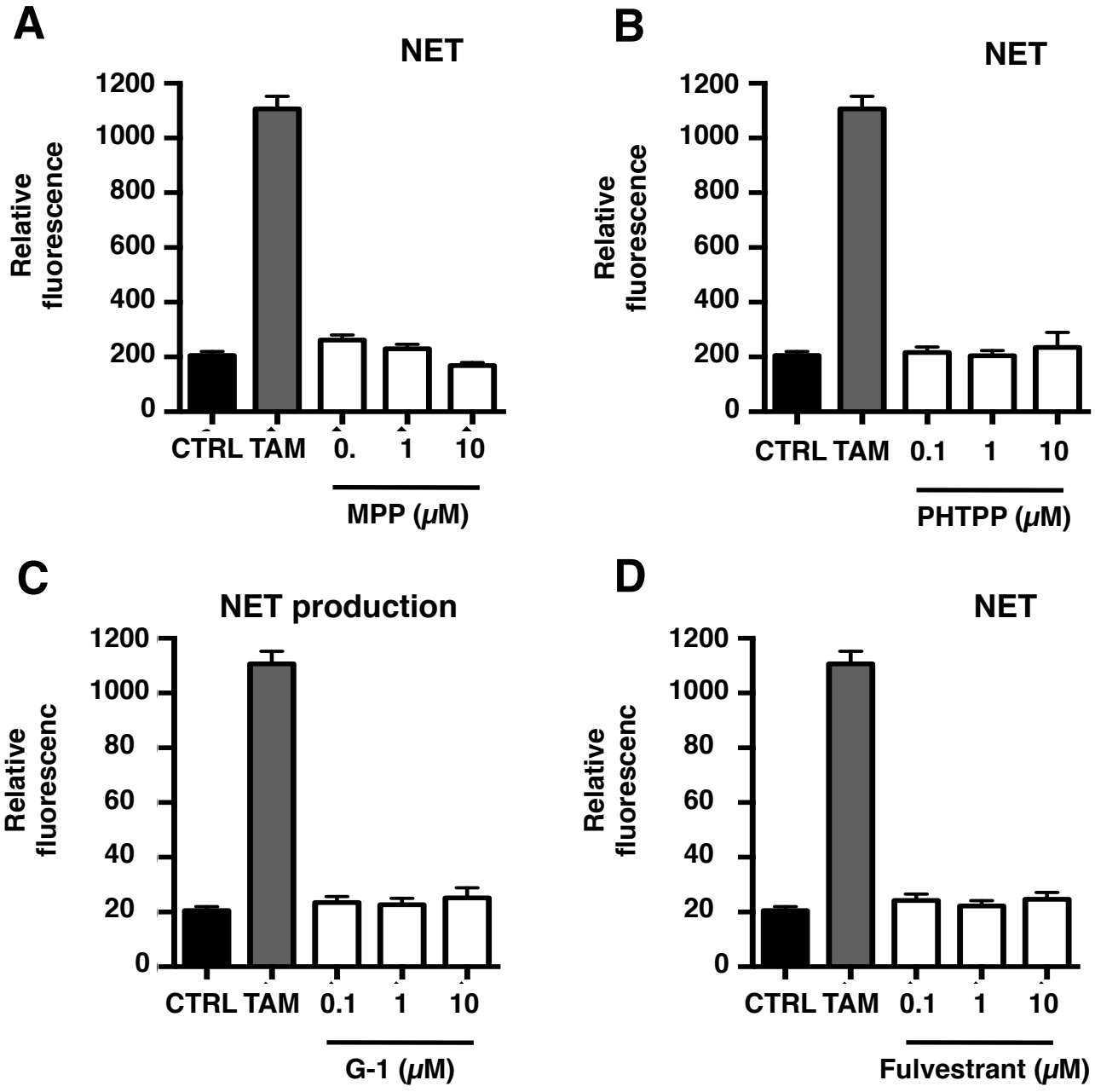


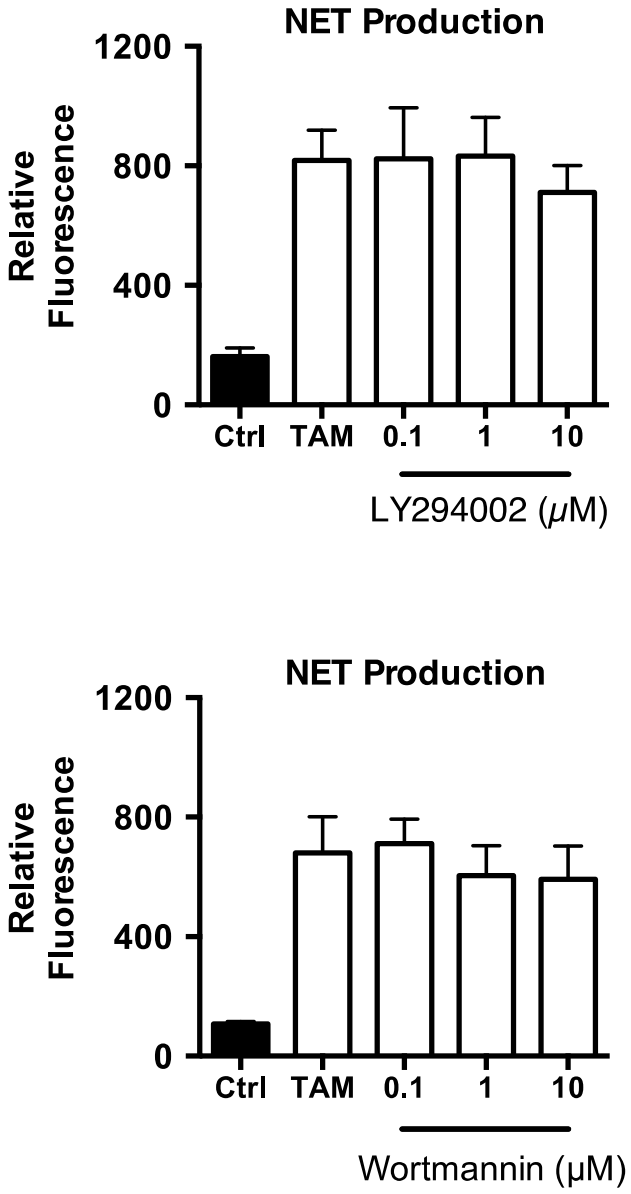
Supplementary Figure 1. Tamoxifen does not promote chemotaxis or chemokinesis in the absence of additional stimulation. Transwell chemotaxis assays revealed that tamoxifen treatment alone did not stimulate migration through a porous filter. N-Formyl-Met-Leu-Phe (fMLP), a potent stimulator of chemotaxis,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



Supplementary Figure 2. N-Desmethyltamoxifen (DTAM) stimulates NET production. Human neutrophils were treated with DTAM (10 μM) 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).

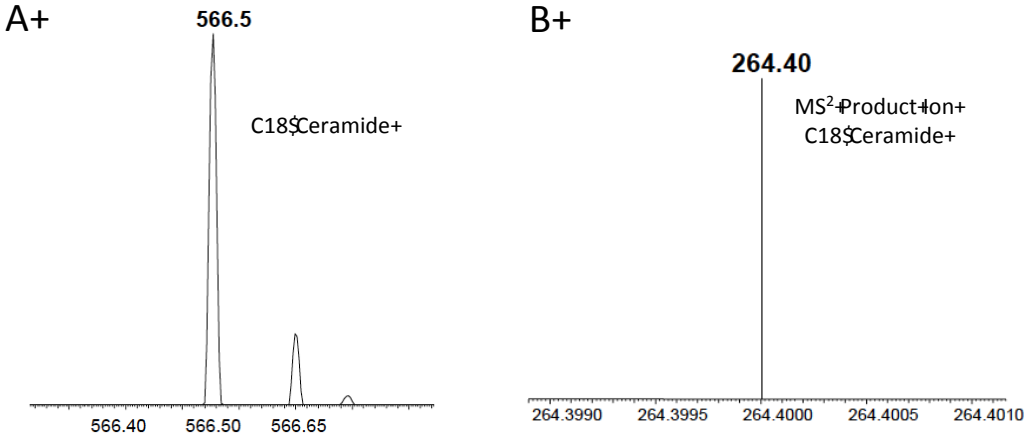


Supplementary Figure 3. Selective estrogen receptor agonists/antagonists do not stimulate NET production. Human neutrophils were treated with MPP (an ERα antagonist), PHTPP (an ERβ antagonist), G-1 (a GPR30 agonist) or fulvestrant (a non-selective SERM that mimics the estrogen receptor effects of tamoxifen)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

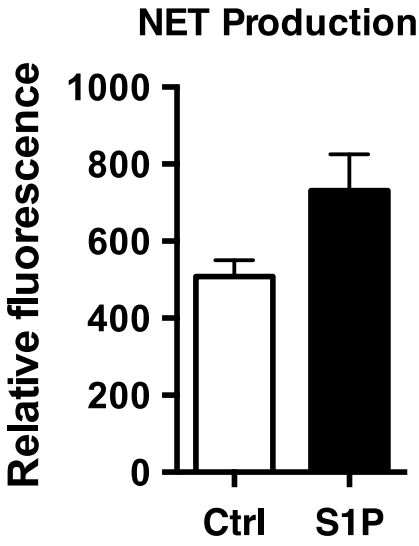


Supplementary Figure 4. PI3K inhibitors do not affect tamoxifen-induced NET production. Human neutrophils were preincubated with the PI3K inhibitors LY294002 or wortmannin for 1 hr at 37ºC with 5% CO_2 prior to addition of 10 μM tamoxifen. NET production was assessed after a further 2 hr incubation under the same conditions (n = 9 for each condition; $p < 0.05$ was taken to represent significance). Neither LY294003 or wortmannin significantly inhibited tamoxifen-

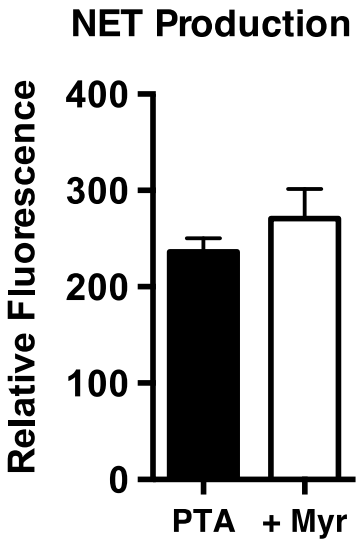
induced NET production. Results were analyzed by one-way ANOVA and post hoc Newman Keuls test.



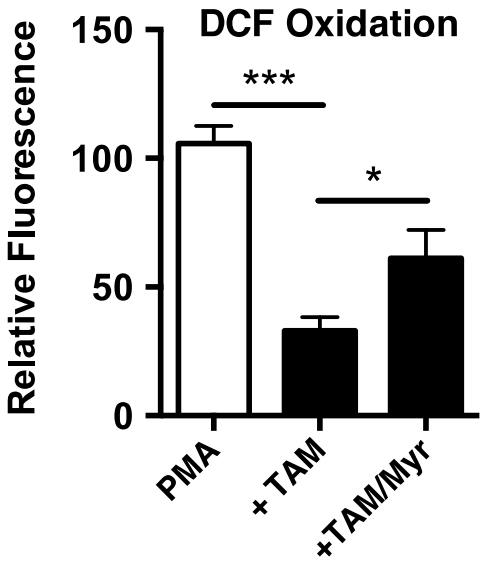
Supplementary Figure 5. Confirmation of presence of ceramide species by MS2 ion verification. (A) Ceramide species were identified by the detection of precursor mono-isotopic masses consistent with previously reported values. (B) The presence of each ceramide was confirmed by the detection of a peak 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.



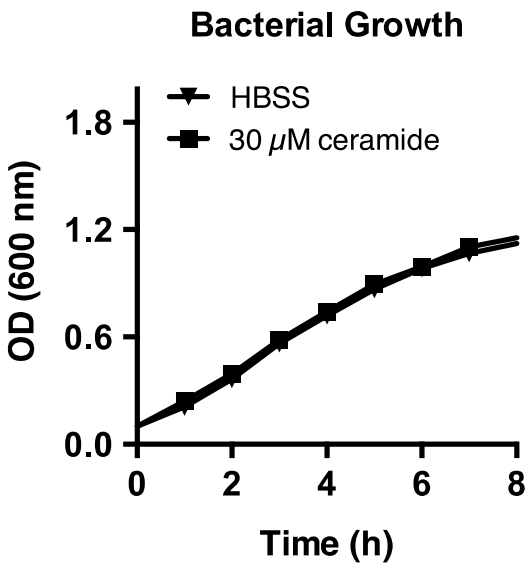
Supplementary Figure 6. Sphingosine-1-phosphate (S1P) does not significantly induce NET production. Human neutrophils were treated with S1P, which is generated from ceramide *in vivo*, for 2 hr at 37ºC. Significant NET production was not observed (n = 9; $p < 0.05$ was taken to represent significance).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

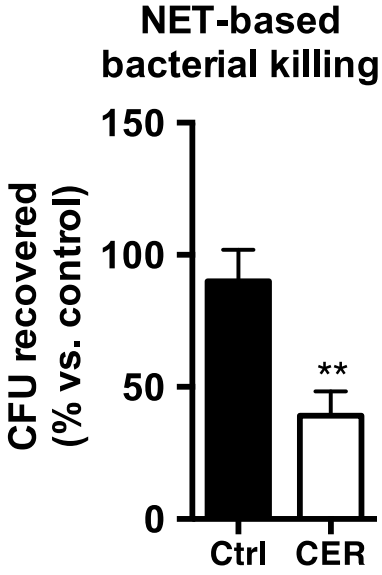


Supplementary Figure 7. Myriocin does not inhibit NET production in response to the PKCζ agonist phosphatidic acid (PTA). To determine whether myriocin directly inhibits PKCζ activity, neutrophils were pre-incubated for 90 min with 3 μM myriocin. Cells were subsequently incubated with 10 μM PTA and incubated 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.

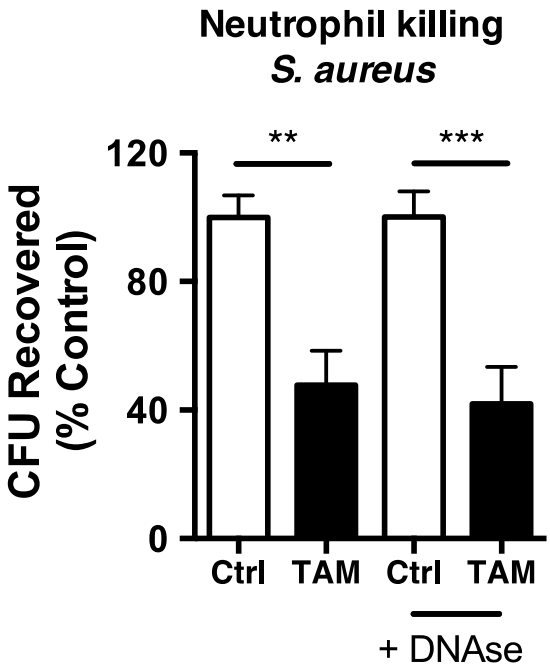


Supplementary Figure 8. Myriocin pre-treatment partially restores ROS production in tamoxifen-treated human neutrophils. After incubation with the fluorescent ROS probe DCF-AM (as described in Methods), human neutrophils were incubated for 90 min at 37C with 5% CO_2 in the presence or absence of 10 μM 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).

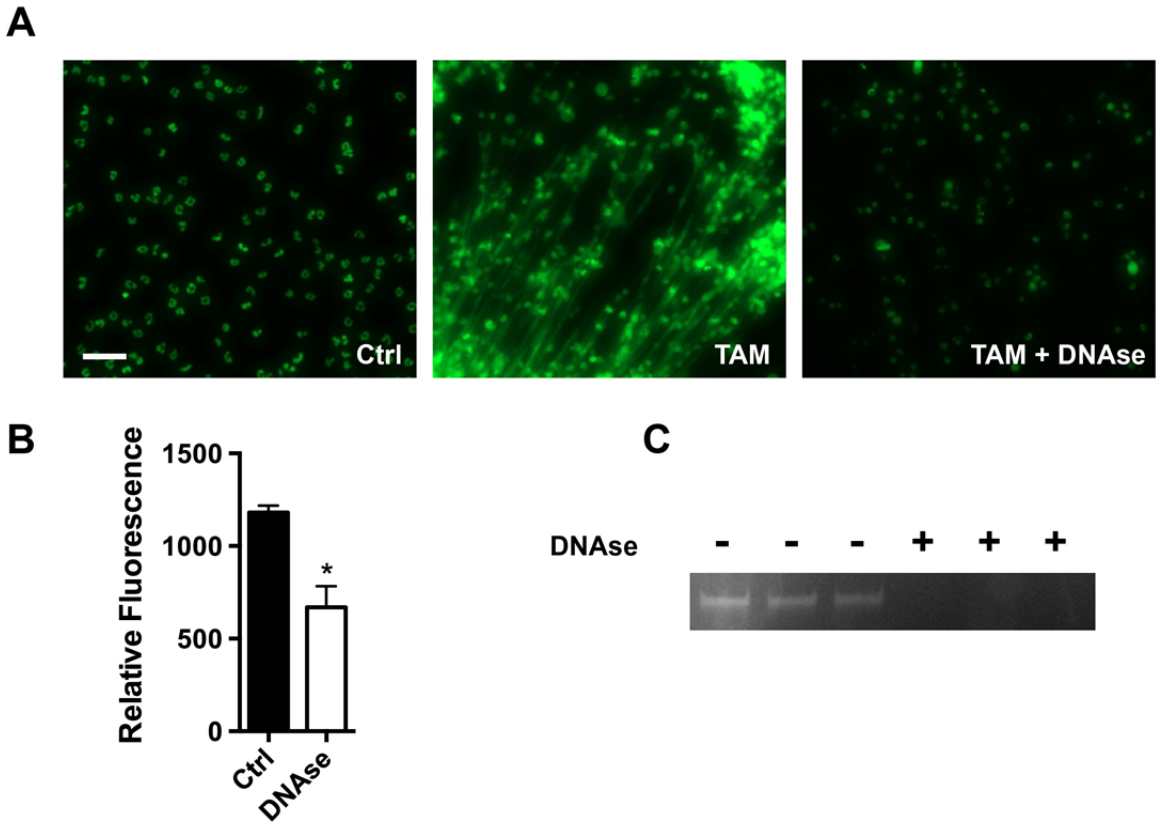




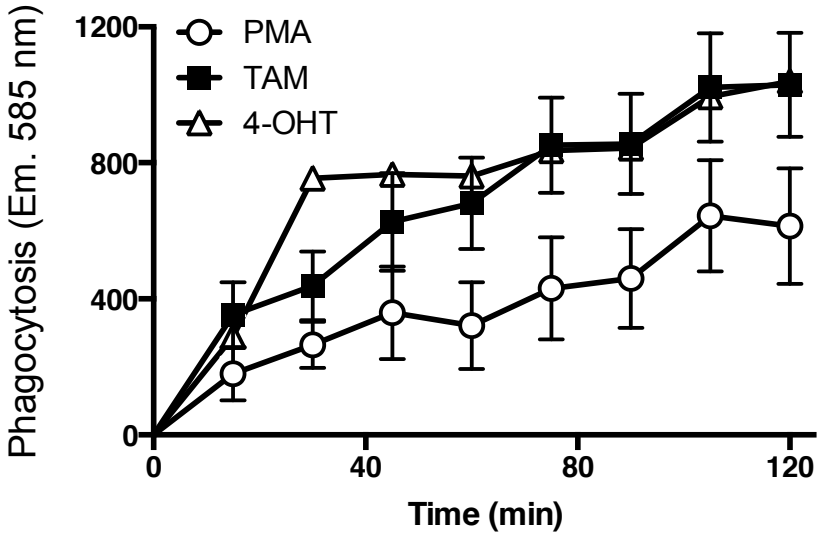
Supplementary Figure 9. Ceramide does not effect growth of bacteria but enhances NET-induced killing. Like Tamoxifen and 4-hydroxytamoxifen, ceramide (30 μM) had no effect on the growth of *Pseudomonas aeruginosa* (n = 6); a significant reduction in bacterial survival was observed in a NET-based bacterial 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 (**$P < 0.01$).



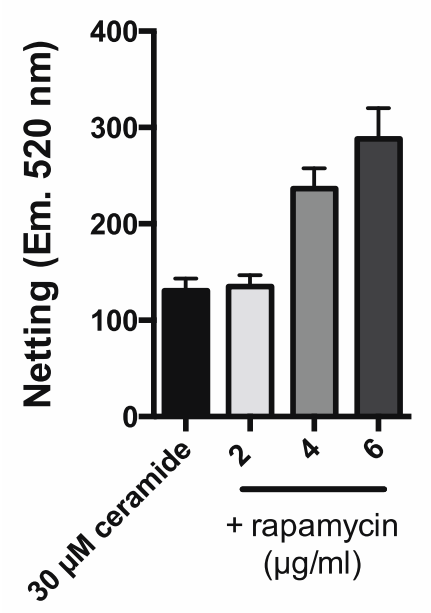
Supplementary Figure 10. Reductions in bacterial CFUs in NET-based killing assays are due to bacterial killing rather than clumping. NET-based killing assays were performed with a USA300 strain of methicillin-resistant *Staphylococcus aureus* as described in Methods; at the initial serial dilution step, 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).



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 μM 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 μm). **(B)** Lambda DNA (2.5 μg) was incubated with DNAse (the same aliquots used for the killing assays) for 30 min at room temperature; intact lambda DNA was quanitifed 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 μM) 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).