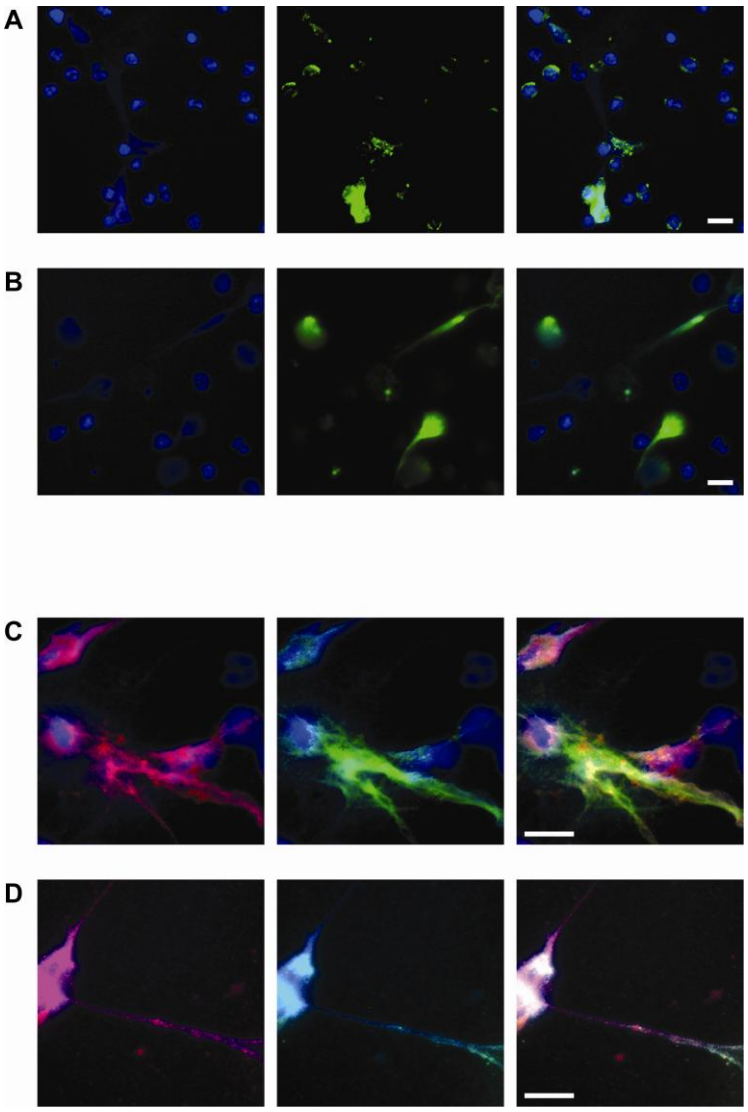
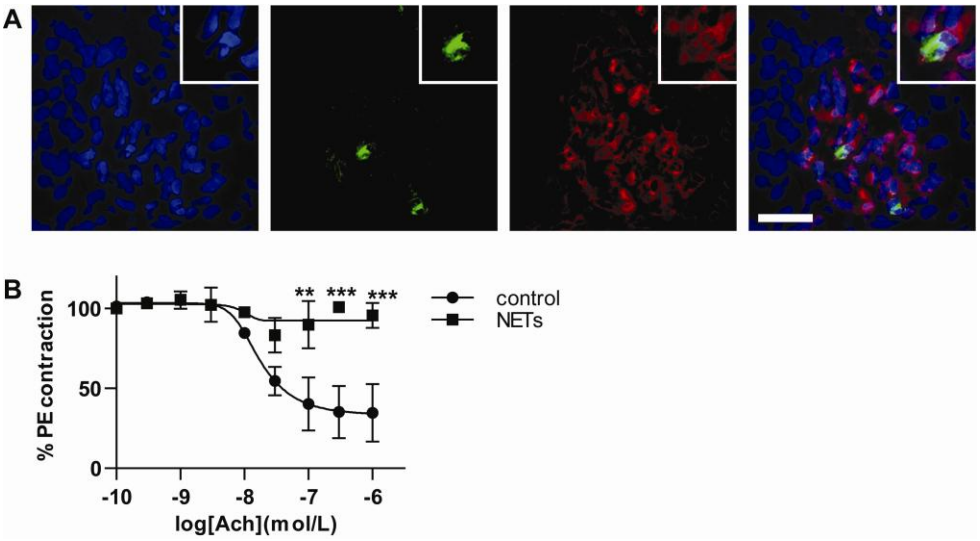
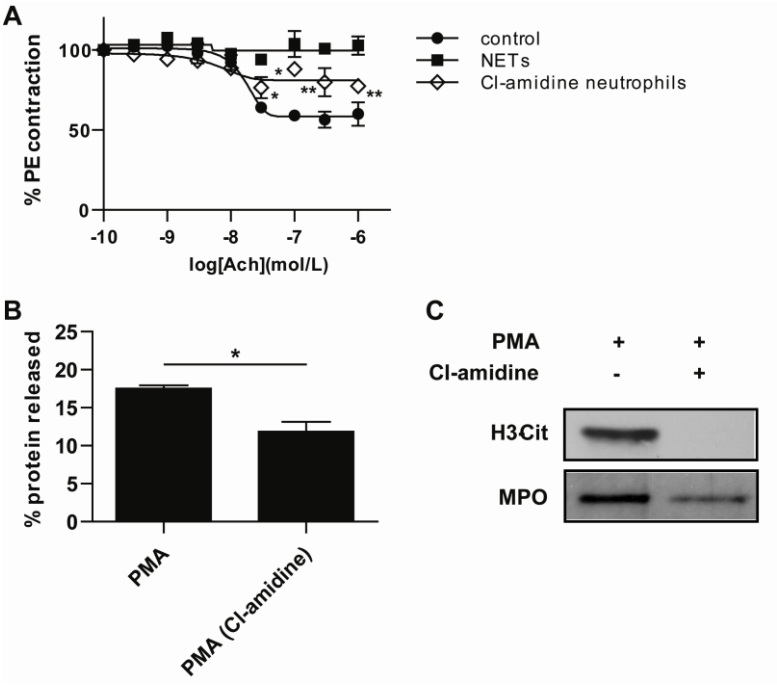


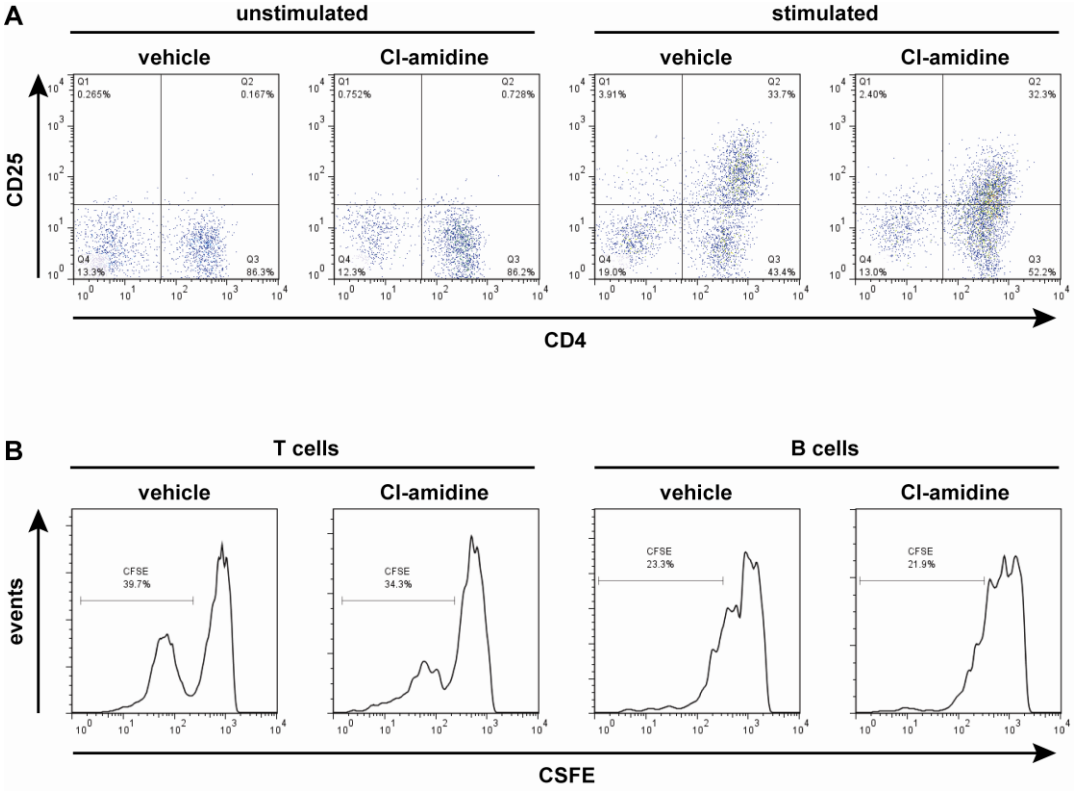
Supplementary Figure 1. NZM NETs contain neutrophil elastase, citrullinated histone H3, and myeloperoxidase; and stain with NZM serum. **A** and **B.** NZM NETs were stained with Hoechst 33342 (blue) and either anti-neutrophil elastase **(A)** or anti-citrullinated histone H3 **(B)**, both in green (original magnification = 400x); overlay images are shown to the far right. **C** and **D.** PMA-induced NETs were stained with 1% NZM serum (detected by Texas Red-conjugated anti-mouse secondary) and either anti-neutrophil elastase **(C)** or anti-MPO **(D)**, both in green (original magnification = 1000x); again, DNA is stained blue and overlay images are shown to the far right. All scale bars represent 10 microns.



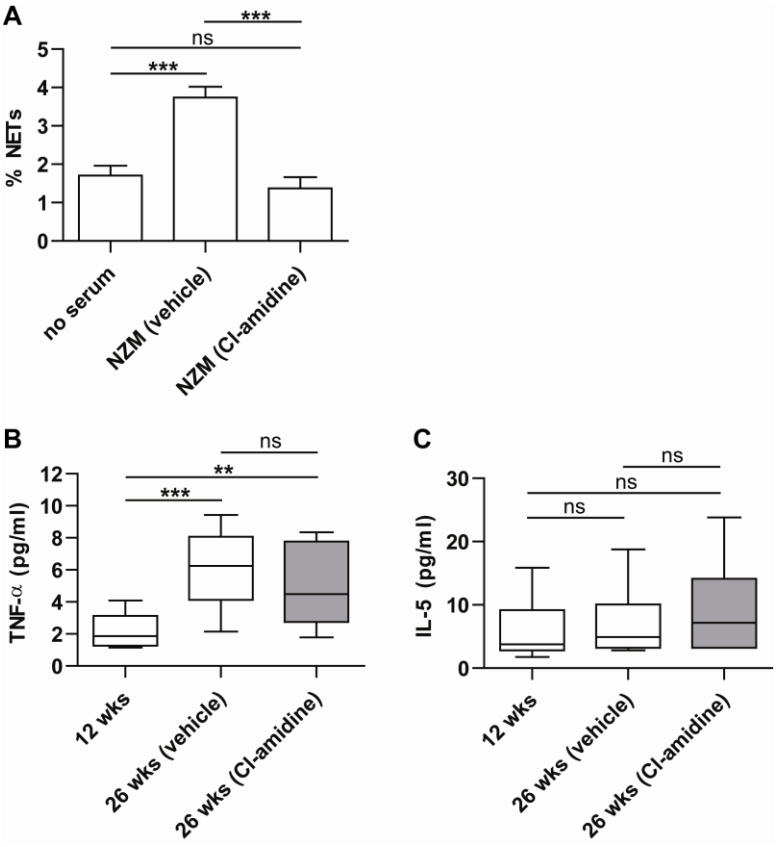
Supplementary Figure 2. NETs can be detected in affected kidneys of NZM mice, and cause endothelial dysfunction in vitro. **A.** Representative MPO-DNA colocalization is shown in kidney sections from proteinuric NZM mice with DNA stained blue, MPO green, and mouse IgG red. An overlay is shown to the right, and highlights an area of extracellular DNA/MPO overlap. Original magnification is 400x, and the scale bar represents 25 microns. **B.** Aortic rings were prepared from young, non-proteinuric NZM mice. Incubation of the rings with NETs (solid squares) inhibited normal endothelium-dependent vasorelaxation when compared to rings not exposed to NETs (solid circles). Each data point is the mean ± SEM for 3 independent experiments. **p<0.01; ***p<0.001.



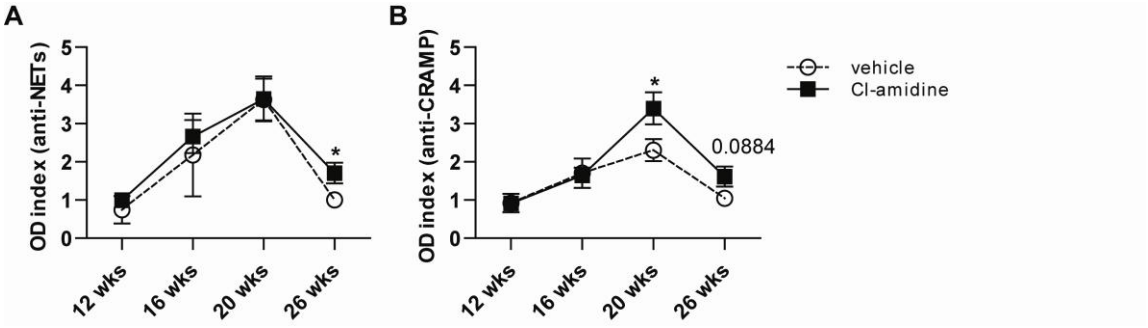
Supplementary Figure 3. NZM neutrophils treated with Cl-amidine in vitro release fewer NETs to damage aortic rings. A. NZM neutrophils isolated from bone marrow were treated with either vehicle or 200 µM Cl-amidine, and then stimulated to release NETs with 100 nM PMA. NETs were isolated and incubated with aortic rings. Endothelium-dependent vasorelaxation of aortic rings was then determined as in Methods. Control = neutrophils treated with no PMA and no Cl-amidine. NETs = neutrophils treated with PMA, but no Cl-amidine. Cl-amidine neutrophils = neutrophils treated with both PMA and Cl-amidine. P values compare Cl-amidine-treated neutrophils to the NETs sample. **B** and **C.** Upon PMA stimulation, NZM neutrophils treated with Cl-amidine release less total protein **(B)**, citrullinated histone H3 **(C),** and myeloperoxidase (MPO) **(C)**. Released protein is compared to corresponding whole cell lysates. Data points represent the mean ± SEM for 3 independent experiments. *p<0.05; **p<0.01.



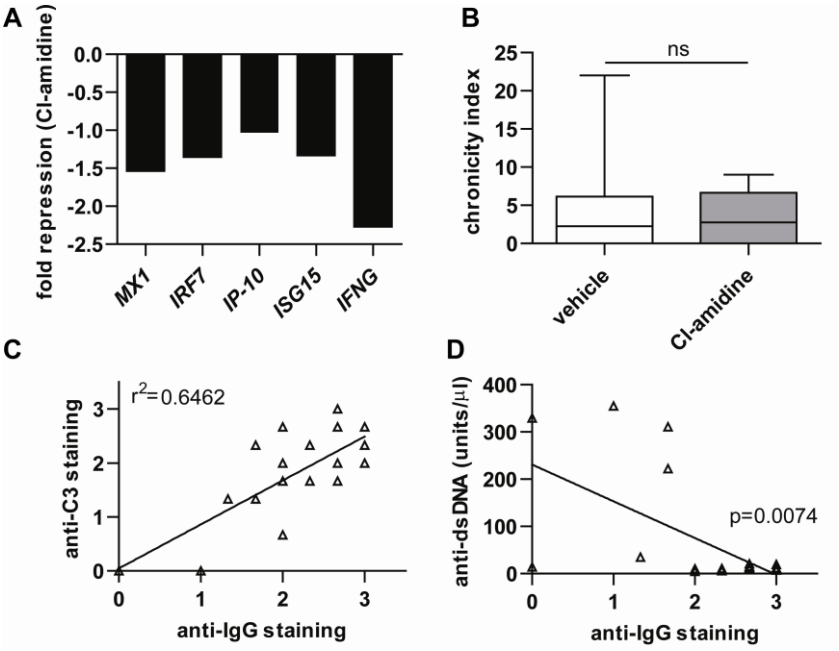
Supplementary Figure 4. Cl-amidine does not affect T cell activation or lymphocyte proliferation in vitro. A. T cells were purified from NZM spleens and stimulated with anti-CD3/CD28 in the presence or absence of Cl-amidine, as described in Methods. CD25-positive cells were quantified by FACS. These plots are representative of at least 3 independent experiments. **B.** T or B cells were purified from NZM spleens, labeled with CFSE, and stimulated with anti-CD3/CD28 or with LPS, respectively. After 48-72 h, in the presence or absence of Cl-amidine, proliferating cells were quantified as described in Methods. These plots are representative of at least 3 independent experiments.



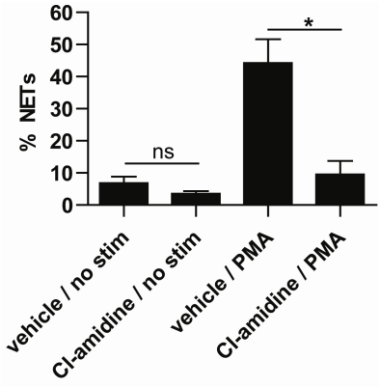
Supplementary Figure 5. Cl-amidine blocks the Induction of NETosis by NZM serum in vivo, but does not have an effect on serum TNF-α or IL-5 levels. A. BALB/c neutrophils were incubated with 2% NZM serum (from the 2 groups of 26-week-old mice presented in Figure 4) for 4 h. Data is presented as the mean ± SEM for 10 mice per group. **B** and **C.** Serum TNF-α and IL-5 levels were measured by multiplex assay as described in Methods for the same 2 groups of 26-week-old mice, and compared to serum from untreated 12-week-old NZM mice. Box-and-whisker plots represent 10 mice per group, with boxes representing the median, 25th percentile and 75th percentile, while whiskers delineate the minimum and maximum values. **p<0.01; ***p<0.001; ns = not significant. Other cytokines were below limit of detection for serum NZM mice.



Supplementary Figure 6. Cl-amidine alters anti-NETs and anti-CRAMP autoantibody levels in vivo. **A** and **B.** Anti-NETs autoantibodies **(A)**, and anti-CRAMP autoantibodies **(B)** were determined by ELISA for the 2 groups of mice presented in Figure 4. Data is presented as the mean ± SEM for 10 mice per group. *p<0.05; one p value that approach significance is presented as a number.



Supplementary Figure 7. Characterization of the type I IFN signature, glomerular chronicity index, and glomerular IgG/C3 deposition in Cl-amidine-treated NZM mice. A. RNA was prepared from the spleens of the aforementioned 26-week-old mice presented in Figure 4, and subjected to quantitative PCR as described in Methods. The data is plotted from the perspective of vehicle-treated mice as the control group; none of the p values reached statistical significance. **B.** Three-micron sections were prepared from formalin-fixed kidneys of the same groups of 26-week-old NZM mice. Chronicity index was calculated as described in Methods. Box-and-whisker plots represent 10 mice per group, with boxes representing the median, 25th percentile and 75th percentile, while whiskers delineate the minimum and maximum values; ns = not significant. **C.** There is a strong correlation between anti-IgG staining and anti-C3 staining within individual kidneys. This data represents all 20 kidneys from the 2 groups of mice described in Figure 4. **D.** Anti-IgG staining is inversely correlated with anti-dsDNA antibody level; again, this data represents all 20 mice from the 2 groups of mice described in Figure 4.



Supplementary Figure 8. One-week treatment with Cl-amidine inhibits NETosis, but does not alter anti-dsDNA level. Bone marrow neutrophils were isolated and incubated either without serum for 4 hours (no stim) or with 100 nM PMA for 12 hours. NETosis was quantified by fluorescence microscopy. Data is presented as the mean ± SEM for 5 mice per group. *$p<0.05$; ns = not significant.