Supplementary Figure Legends

Supplementary Figure 1. Comparison of RNP IC-mediated NET formation. Quantification of DNA release induced by ICs consisting of SmRNP combined with SLE IgG 961 (n = 10), 1032 (n = 6) and 984 (n = 7) or IgG from healthy individuals (HC IgG, n = 7). The bars represent mean + SEM. For statistical analyses, all samples were compared to non-stimulated neutrophils using t-test with ns = non-significant, **P < 0.01 and ***P < 0.001.

Supplementary Figure 2. RNP IC-mediated NETosis requires mitochondrial ROS. Immunofluorescence microscopy of RNP IC-mediated NET formation in neutrophils from healthy individuals in presence of ROS inhibitors. The images are representative of 3 independent experiments.

Supplementary Figure 3. RNP IC-mediated mitochondrial extrusion from neutrophils. (a) Flow cytometry analysis of cell surface TOM20 levels in live (propidium iodide-negative) neutrophils upon RNP IC activation. (b) Quantification of 16S DNA levels in cells and NETs upon RNP IC activation. The results are normalized to non-stimulated cells. For statistical analyses, paired t-test was used with *P < 0.05, *P < 0.01 and **P < 0.001.

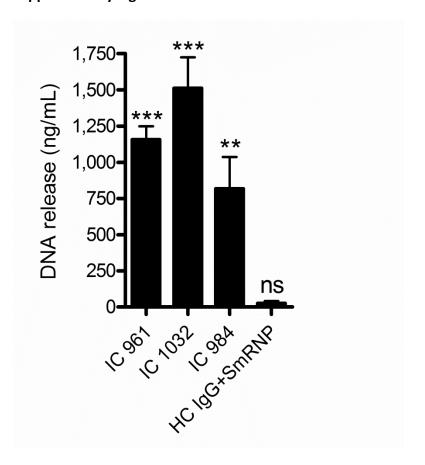
Supplementary Figure 4. Interferogenic ability of DNA is dependent on oxidation. DNA, isolated from nuclei (nuc) or mitochondria (mito) from several different species and cell types (HT: herring testes, mouse heart and spleen, Jurkat cells, human neutrophils (PMNs) and peripheral blood mononuclear cells (PBMCs)), were either exposed to UV-mediated oxidation (+) or not (-) and analyzed for 8-OHdG content (a, c) and interferogenic ability upon transfection into THP1 cells (b, d and e). Figures a–d are representative from individual experiments, and figure (e) is compiled data from n=7 paired samples. For statistical analyses, Spearman's correlation (b), Pearson's correlation (d) and paired t-test (e) were used with ns = non-significant, *P < 0.05, **P < 0.01 and *** P < 0.001.

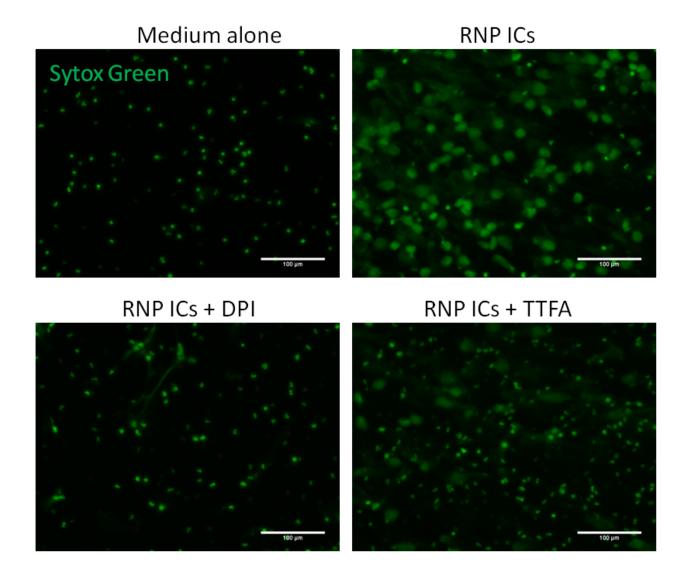
Supplementary Figure 5. 8-OHdG⁺ DNA ICs do not activate neutrophils. Neutrophil activation by DNA-containing ICs was analyzed by (a) CD66b cell surface expression (n = 3), (b) NET formation (n = 2), (c-e) IC binding and phagocytosis by flow cytometry (n = 4) as well as by (f) fluorescence microscopy (n = 2). For statistical analyses, paired t-test was used with *P < 0.05, **P < 0.01 and *** P < 0.001.

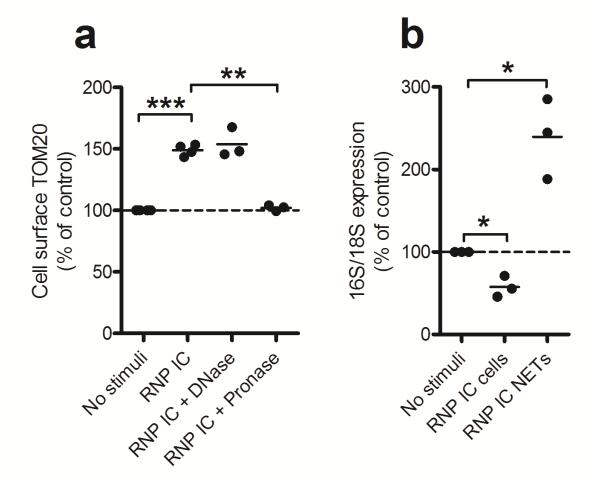
Supplementary Figure 6. SLE patients have serologic evidence of ongoing NETosis. Plasma samples from healthy controls (n = 20) and SLE patients (n = 20) were analyzed for markers of

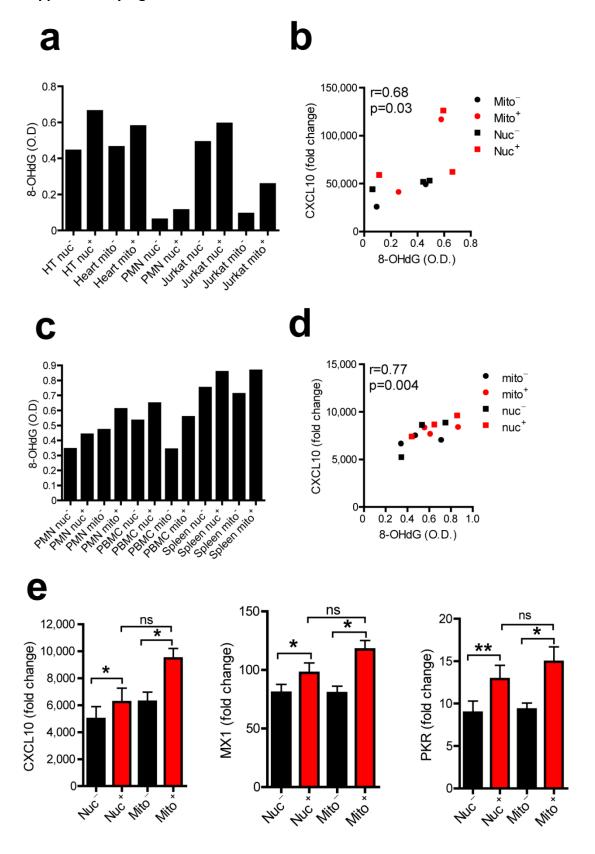
NET formation; (a) citrullinated histone H4:MPO complexes, (b) DNA:human neutrophil elastase complexes and (c) DNA:MPO complexes. (d) Correlation between serum peroxidase activity and SLEDAI-2K (n=16). For statistical analyses, 2-sided unpaired t-test (a) and (c), 2-sided unpaired Mann-Whitney test (b) and Spearman's correlation test (d) were used with *P < 0.05, **P < 0.01 and *** P < 0.001.

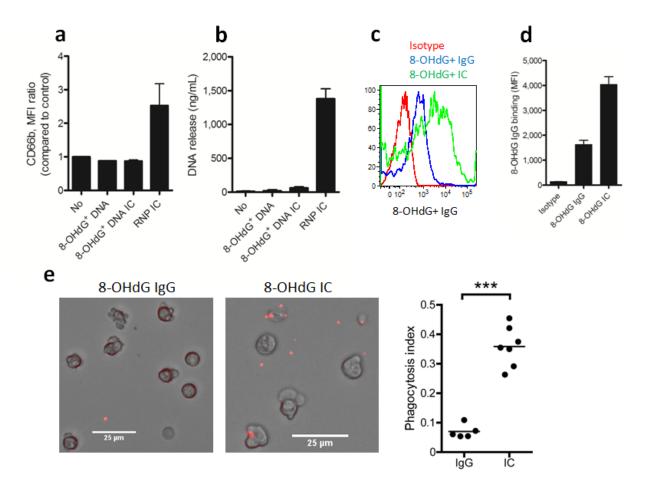
Supplementary Figure 7. CGD patients have a type I IFN signature. Quantification of ISG induction by healthy control and CGD sera (n = 15 and 22 respectively). The results are expressed as fold induction index (mean \pm SEM) as compared to healthy control sera. For statistical analyses, unpaired Mann-Whitney test was used with *P < 0.05 and **** P < 0.0001.

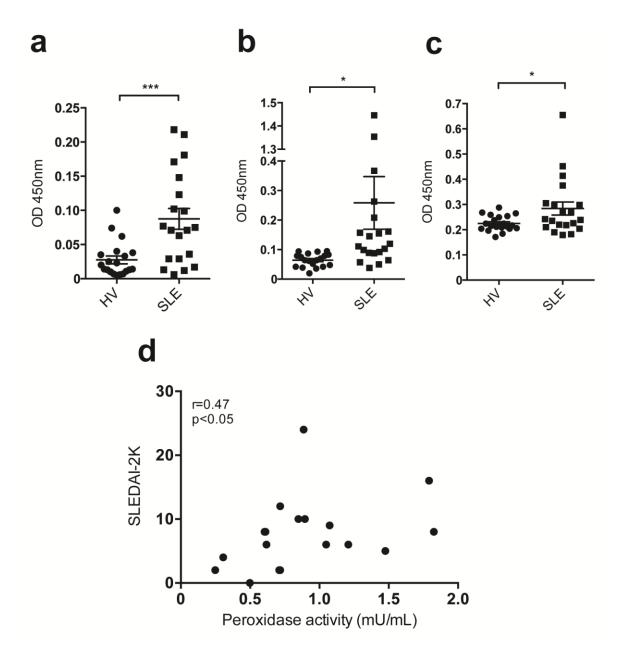


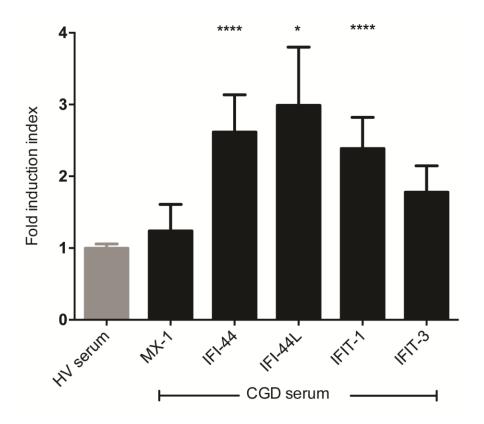












Supplementary Table I. Demographics and clinical characteristics of CGD patients (n=22) at enrollment

Characteristics	Percentage
Male	86.4
Mean age, years (SEM)	30.0 (1.7)
p47 mutation (NCF1)	59.1
gp91 mutation (CYBB and CYBA)	40.9
X-linked mutations	63.6
Autosomal recessive mutations	36.4
Clinical autoimmunity	68.2
- Inflammatory bowel disease	13.6
- Cutaneous or systemic lupus	13.6
- Vasculitis	4.5
- Celiac disease	4.5
- Type-I diabetes	4.5
- Psoriasis	4.5
- Sarcoidosis	4.5
- Juvenile idiopathic arthritis	4.5
Immunosuppression	59.1
- Prednisone	59.1
- Azathioprine	9.1
- Methotrexate	4.5

- 6-Mercaptopurine	4.5
- Hydroxychloroquine	4.5
- Anakinra	4.5
Hypocomplementemia	18.2
ANA, anti-ENA and/or anti-dsDNA	36.4
Rheumatoid factor	13.6
Anti-CCP	9.1
aPL antibodies	13.6
LDGs/mL blood (mean \pm SEM)	238,365 ± 95,630
Superoxide residual activity *	2.12 ± 1.21
- p47 mutation	2.90 ± 0.64
- gp91 mutation	1.04 ± 1.33

Abbreviations used in the table: CGD: chronic granulomatous disease; NCF1: neutrophil cytosolic factor1; CYBB: cytochrome b-245, alpha polypeptide; CYBA: cytochrome b-245, alpha polypeptide; ANA: antinuclear antibody; ENA; anti-extractable nuclear antigen antibody (includes Ro, LA, Sm, SmRNP, RNP); dsDNA: double-stranded DNA; ANCA: antineutrophil cytoplasmic antibody (includes anti-myeloperoxidase, and anti-proteinase-3 antibodies); CCP: anti-cyclic citrullinated peptide Antibodies; aPL: anti-phospholipid antibodies (includes lupus anticoagulant, anti-cardiolipin antibodies and beta-2 glycoprotein antibodies). * Extracellular superoxide residual activity is quantified by the cytochrome C assay as nmol per 10^6 cells per hour and represents mean \pm SD of all CGD patients and also divided by type of mutation. As reference, normal superoxide production is 222.6 ± 3 nmol/ 10^6 cells per hour.