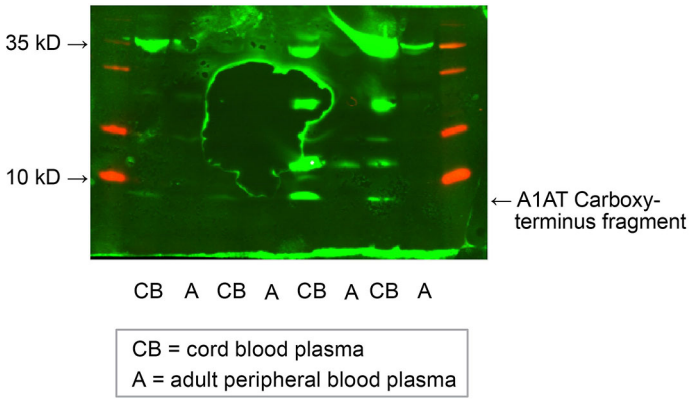
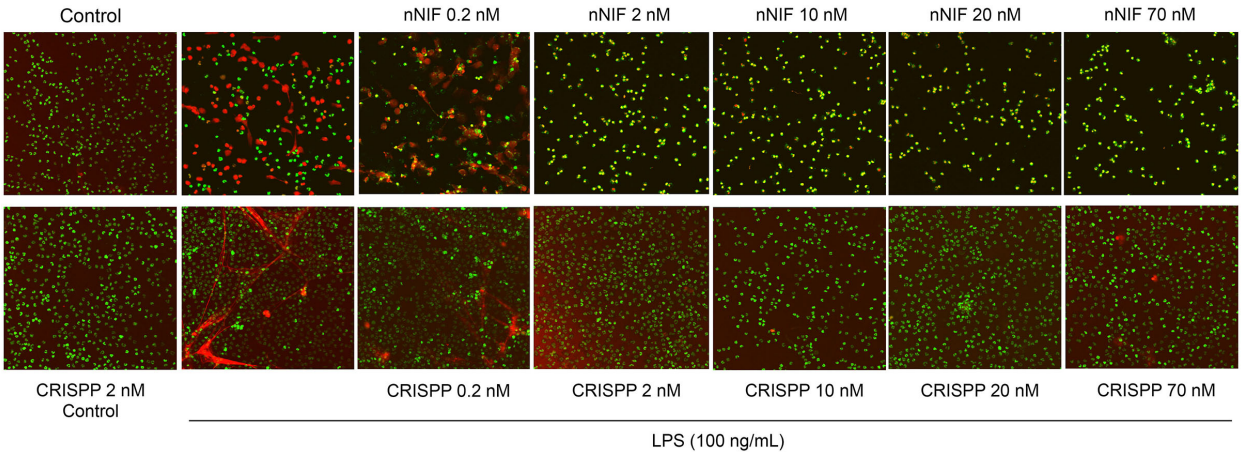


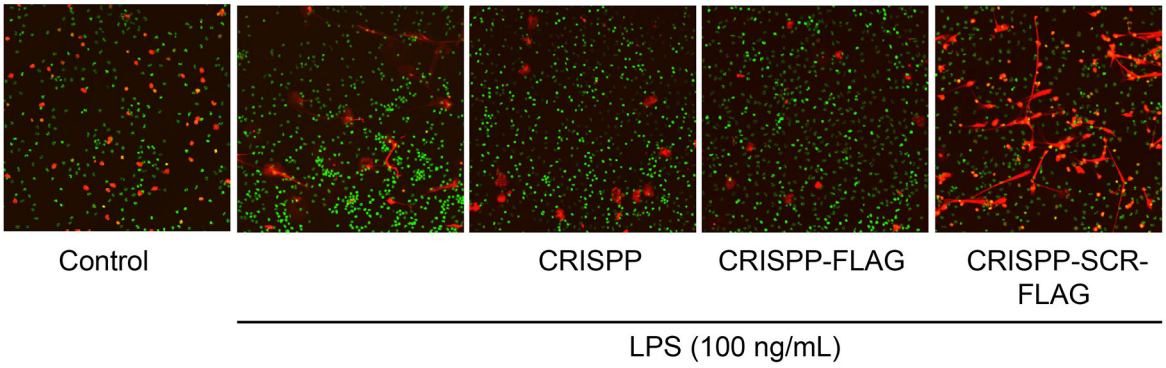
Supplemental Figure 1. A low molecular weight peptide recognized by an antibody against the carboxy-terminus of A1AT is detected in umbilical cord blood samples but not plasma from adults.

Samples of cord blood plasma from 4 healthy term neonates and venous blood samples from 4 healthy adult volunteers were examined by Western blotting. This is the full gel from which the left panel of Fig. 2B was prepared.



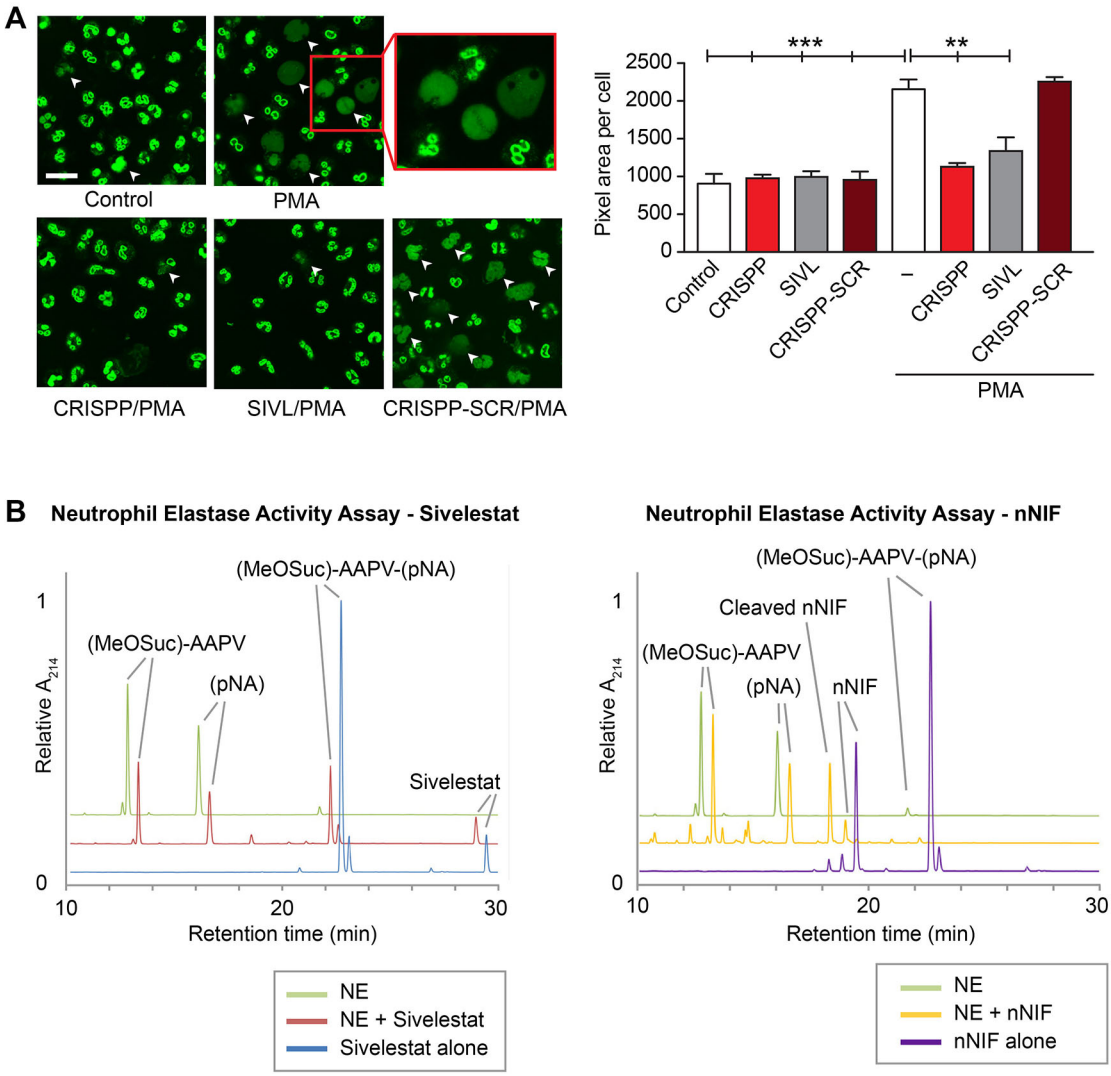
Supplemental Figure 2. nNIF and CRISPP inhibit NET formation at nanomolar concentrations.

PMNs from healthy adult volunteers were preincubated in medium alone or with nNIF or CRISPP in the indicated concentrations for 1hr. LPS (100 ng/mL) was then added and the leukocytes were incubated for 1hr followed by live cell imaging as in text **Fig. 1A** (red fluorescence = NETs; green fluorescence = nuclear DNA; 20x magnification). This concentration-dependent inhibition of NET formation by nNIF and CRISPP was seen in three experiments with neutrophils from different donors.



Supplemental Figure 3. FLAG-tagged CRISPP inhibits NET formation by LPS-activated neutrophils.

Adult neutrophils were preincubated for 30 minutes in medium alone or with CRISPP, FLAG-tagged CRISPP (CRISPP-FLAG), or FLAG-tagged CRISPP-SCR (CRISPP-SCR-FLAG) (1nM for all), stimulated with LPS (100ng/mL), and incubated for 1hr followed by live cell imaging as in text **Fig. 1A** to assess NET formation (red fluorescence =NETs; green fluorescence = nuclear DNA; 20x magnification). Inhibition of NET formation by CRISPP-FLAG and CRISPP but not CRISPP-SCR-FLAG was seen in three separate experiments with neutrophils from different donors.



Supplemental Figure 4. Neutrophil elastase (NE) mediates nuclear decondensation but nNIF and CRISPP do not inhibit NE activity *in vitro*.

(A) Nuclear decondensation (white arrowheads and magnified image) was assessed as in text **Fig. 7** after a 1hr preincubation in medium alone, with the NE inhibitor sivelestat (SIVL; 200nM), or with CRISPP or CRISPP-SCR (both 1nM) followed by treatment with PMA (20nM) and an additional 1hr incubation (*n*=3). Green fluorescence = nuclear DNA, (60x magnification, scale bar = 20µm). Nuclear area was quantified using ImageJ software (nuclear pixel area per cell ± SEM). One way ANOVA with Tukey's post hoc testing; ***P<0.01*, ***P<0.001. **(B)** NE enzyme activity was examined by cleavage of the synthetic substrate (MeOSuc) – AAPV – (pNA) to (MeOSuc) – AAPV and (pNA) as products detected by liquid chromatography and chromatogram peak identification by mass spectroscopy. (MeOSuc) – AAPV – (pNA) (160µm) was incubated with NE (2mU), sivelestat (160µm), or NE and sivelestat (**left panel**) or with NE (2mU), nNIF (10nM), or NE and nNIF (**right panel**) for 3hrs at 37°C. Chromatograms are offset on the X and Y axes for ease of comparison. In the presence of NE alone the (MeOSvc) – AAPV – (pNA) peak was almost completely eliminated and (MeOSvc) – AAPV and (pNA) peaks were generated. In the presence of sivelestat this substrate cleavage was inhibited but not eliminated. In contrast, it was not inhibited by nNIF. This pattern was seen in three separate experiments. Using two additional assays

employing different protocols, NE substrates, and detection methods, neither nNIF nor CRISPP inhibited NE activity in multiple experiments (unpublished data).

Supplemental Table 1. Clinical characteristics and infectious complications of preterm infant subjects

Gestational ages at birth	$23^{6}/_{7} – 29^{0}/_{7}$ weeks
Birth weight	570 – 1160 g
Female gender	55%
Indication for pre-term delivery	
Prolonged premature rupture of membranes or preterm labor	8
Pregnancy induced hypertension	1
Placental abruption/preterm labor	0
Bacterial blood culture results	
E. coli	0
Coagulase (–) *Staphlococcus*	2
Group B Streptococcus	0
Negative	6
Meningitis	2
Pneumonia	2
Antibiotic treatment	All treated, 2-14 d

Supplementary Table 2. Sequences for the NET-inhibitory peptides and their specific scrambled peptide controls

nNIF	KFNKPFVFLMIEQNTKSPLFMGKVVNPTQ
nNIF-SCR	LNTNKTKMGVQFPKMPFFKQIPVNSLEFV
CRISPP	M_IPPEVKFNKPFVFLMIDQNTKVPLFMGK
CRISPP-SCR	V_MDITPMQVGPLKMKPKVIFNPFKLFENF
A1ATm^{358}	MFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMLKVVS
A1ATm^{358}-SCR	PMVSVAMMLSENIFKLPEVKSVPTEFFPKFINMKLLPFQI