www.sciencetranslationalmedicine.org/cgi/content/full/3/73/73ra19/DC1

Supplementary Materials for

Neutrophils Activate Plasmacytoid Dendritic Cells by Releasing Self-DNA–Peptide Complexes in Systemic Lupus Erythematosus

Roberto Lande, Dipyaman Ganguly, Valeria Facchinetti, Loredana Frasca, Curdin Conrad, Josh Gregorio, Stephan Meller, Georgios Chamilos, Rosalie Sebasigari, Valeria Riccieri, Roland Bassett, Hideki Amuro, Shirou Fukuhara, Tomoki Ito, Yong-Jun Liu, Michel Gilliet*

*To whom correspondence should be addressed. E-mail: michel.gilliet@chuv.ch

Published 9 March 2011, *Sci. Transl. Med.* **3**, 73ra19 (2011)
DOI: 10.1126/scitranslmed.3001180

The PDF file includes:

Reference

SUPPLEMENTAL MATERIAL AND METHODS

Reagents. The synthetic peptide LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES) was purchased from Innovagen (Lund, Sweden). The natural human neutrophil peptides [$HNP_{(1-3)}$] were from Hycult Biotechnology (Canton, MA). Synthetic phosphothioate TLR9 agonist CpG-ODN 2006 (5'-tcgtcgttttgtcgttttgtcgtt-3') was produced by TriLink BioTechnologies (San Diego, CA) and was used at 1 μM. Synthetic TLR7 agonist R837 (10 μg ml^{-1}) (imiquimod) was from InvivoGen (San Diego, CA). The specific human TLR9 inhibitor (1 μM) (ODN TTAGGG, 5'-tttagggttagggttagggttaggg-3') was purchased from InvivoGen, and the control ODN sequence (1 μM) (5'-tcctgcaggttaagt-3') was a kind gift from Frank Barrat (Dynavax Technologies, Berkeley, CA). DNase I (800 U ml^{-1}) was from Roche (Nutley, NJ). Human IFNα-2b was from Schering Plough (Segrate, Milan, Italy) and was used at 100 U/ml. Monoclonal antibody against LL-37 (clone 8A8, IgG2b) was generated by immunizing 6- to 8-wk-old BALB/c mice with synthetic LL-37 peptide and was used at 10 μg ml^{-1}. Monoclonal antibody against HNPs (clone 3G9, IgG2a) was from Novus Biological (Littleton, CO) and was used at 10 μg ml^{-1}. Monoclonal antibody against dsDNA (clone H241, IgG2b,) was a kind gift from Dr. Stollar, Tufts University, and was used at 10 μg ml^{-1}. Neutralizing anti-CD32 antibody (clone AT-10) was from Abcam (Cambridge, MA) was used at 10 μg ml^{-1}. Proteinase 3 inhibitor N-methoxy-succinyl-ala-ala-pro-val chloromethyl ketone (CMK) was from Sigma Aldrich (St. Louis, MO) and was used at 0.1 mM. N-

Acetyl L cysteine (NAC) was from Sigma Aldrich and was used at 5 mM. Agonistic anti-human CD95 antibody (anti-Fas, clone CH11) was from Upstate Biotechnology (Lake Placid, NY) and used at 1 μg ml^{-1}.

LL37 detection by immuno blot. For detection of LL37 by immunoblotting, samples were resolved by electrophoresis on a 16% Tris-Glycin polyacrylamide gel under reducing conditions and transferred onto a polyvinylidene fluoride microporous membrane (Immobilon-P Transfer Membrane; Millipore, Billerica, MA). Membranes were blotted overnight at room temperature with biotinylated rabbit anti-LL37 antibody (Innovagen), followed by incubation with streptavidin-HRP (R&D Systems, Minneapolis, MN). Immunoblotted proteins were visualized by enhanced chemiluminescence (SuperSignal West Dura, Pierce Biotechnology, Rockford, IL). To ensure the disruption of the complexes, IgG-containing samples were pretreated for 40 min at +37°C with Poly-L-aspartic acid (25 mM) and DNase I (30 units) before separation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Nano-HPLC/MS/MS analysis. In-gel digestion and nano-HPLC/MS/MS for peptide identification were carried out as described before [1] . The Coomassie brilliant blue–stained bands (between 4 and 6 kD) were excised and destained with 50 mM ammonium bicarbonate solution in 50% methanol. Gel pieces were then washed in HPLC water overnight. After the wash procedure, gel pieces were

digested with 100 ng of trypsin in 50 mM NH_4HCO_3 (pH 8.5) for 4 h in a volume of 15 µl. After digestion, peptides were extracted by the addition of 200 µl of acetonitrile. The supernatants were dried in a Speed-Vac dryer (Thermo Savant). Each dried sample was dissolved in 20 µl of 5% methanol/95% water/0.01% formic acid solution and injected into a Surveyor HPLC system (ThermoFinnigan) using an autosampler. A 50- x 75-mm C18 column (BioBasic C18, 5 µm, 300 Å pore diameter, PicoFrit, New Objective, Cambridge, MA) with mobile phases of A (0.01% formic acid in water) and B (0.01% formic acid in methanol) was used with a gradient of 5-95% of mobile phase B over 15 min followed by 95% B for 5 min at a flow rate of 200 nl/min. Peptides were directly eletrosprayed into a mass spectrometer (Finnigan LTQ, ThermoFinnigan) with use of a nanospray source. LTQ were operated in the data-dependant mode acquiring fragmentation spectra of the top 20 strongest ions. Obtained MS/MS spectra were analyzed with use of a modified NCBI-ref protein sequence database based on a BioWorks database search engine (BioWorksBrowser ver. 3.2, Thermo Electron). All peptide identification with stringent BioWorksBrowser filtering criteria (peptide probability >1 x 10^{-6} and X corr score >2.0) was manually examined, and all peptides had to be identified by consecutive b- or y- ions so that false identifications could be eliminated.

DNase I protection assay. Self-DNA complexes were treated for 1 h with DNase I (100 U ml^{-1}) or with DNase I-free buffer and stained with PicoGreen for quantitating dsDNA (Quant-iT PicoGreen dsDNA kit, Invitrogen, Carlsbad, CA)

according to the standard protocol provided by the manufacturer. In some experiments, the complexes were pretreated for 30 min with polyAsp (10 μM) or polyGlu (10 μM) (both from Sigma-Aldrich) before DNase I digestion. Samples were excited at 480 nm, and the emission intensity was measured fluorometrically at 520 nm. For NET digestion DNAse was used at 10 U ml^{-1} for 30 minutes.

DNA uptake by pDC. To visualize the uptake of self-DNA by pDCs, human DNA (BioChain, Hayward, CA) was labeled with Alexa488 by using the Ulysis Nucleic Acid Labeling Kit (Molecular Probes, Carlsbad, CA), according to the standard protocol provided by the manufacturer as previously described. Purified pDCs were stimulated for 4 h with $DNA^{Alexa488}$ -containing complexes, washed, and analyzed by flow cytometry.

Immunomagnetic depletion of LL37- and HNP-containing immune complexes. 1-2 mg of purified immune complexes (IgG >300 kD) were incubated for 45 min at room temperature with either a biotinylated anti-LL37 antibody (Innovagen, 30 μg ml^{-1}) or a biotynylated anti-HNP antibody (Hycult Biotechnology, 30 μg ml^{-1}), followed by incubation with streptavidin-microbeads (Miltenyi Biotec) and then passage through a MACS column (Miltenyi Biotec). The negative flow was collected and used to stimulate pDC.

Visual NET quantification. NET release was quantified as NET density on confocal microscopy images acquired at 200x magnification. The percentage of

the image area containing NET structures (defined as bright fluorescence larger in size than a neutrophil) was evaluated by two independent investigators and given as a net density score ranging from 1 to 10: a score of 1 indicates involvement of 0-10% of the image, score 2 indicated involvement of 10-20%, and score 10 indicates involvement of 90-100%. As a confirmatory approach, an automated density score was obtained by analyzing these images with the imageJ software (public software developed by NIH). After applying a fluorescence threshold, this software measures the percentage of pixels with fuorescence among the total pixels of the image.

Detection of serum antibodies to antimicrobial peptides. Serum levels of anti-LL37 and anti-HNP antibodies were measured by ELISA. Briefly, 96-well flat-bottom plates were coated with 1 μg ml^{-1} of LL37 (Innovagen), HNP (Hycult Biotechnology), or HBD2 (Innovagen) in carbonate buffer (0.1 M $NaHCO_3$, pH 9.6) for 2 h and washed four times with PBS containing 0.05% Triton X-100. This washing buffer was used for washing at all steps. The blocking buffer containing 2% BSA (Sigma) in PBS was used for 1 h to saturate unspecific binding sites. After washing, sera were diluted 1:640 in PBS + 2% BSA followed by 1 h of incubation with a horseradish peroxidase–conjugated goat anti-human IgG (Sigma-Aldrich) diluted 1:5000 in PBS. The color was developed for 30 min with use of 3,3',5,5'-tetramethylbenzidine (TMB) substrate (Sigma-Aldrich). The reaction was stopped by adding 50 μl of 0.2 N H_2SO_4, and absorbance was determined at 450 nm with a reference wavelength of 540 nm.

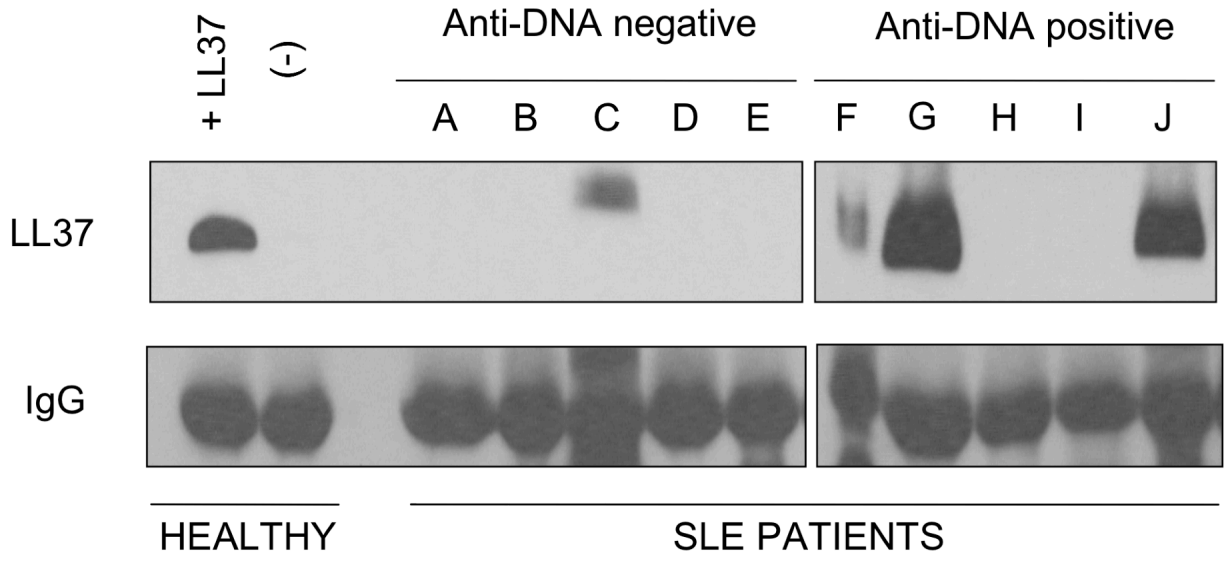

Fig. S1. Detection of LL37 in total IgGs of anti-DNA-positive SLE patients. Immuno blot analysis of total IgG antibodies isolated from sera from 5 SLE patients with high levels of circulating anti-DNA antibodies (> 115 U/ml) and SLE patients with low levels of anti-DNA antibodies (< 100 IU/ml). Total IgGs were separated by sodium dodecyl sulfate gradient gel, and detected with antibodies against LL37 and anti-human IgG Fc antibodies.

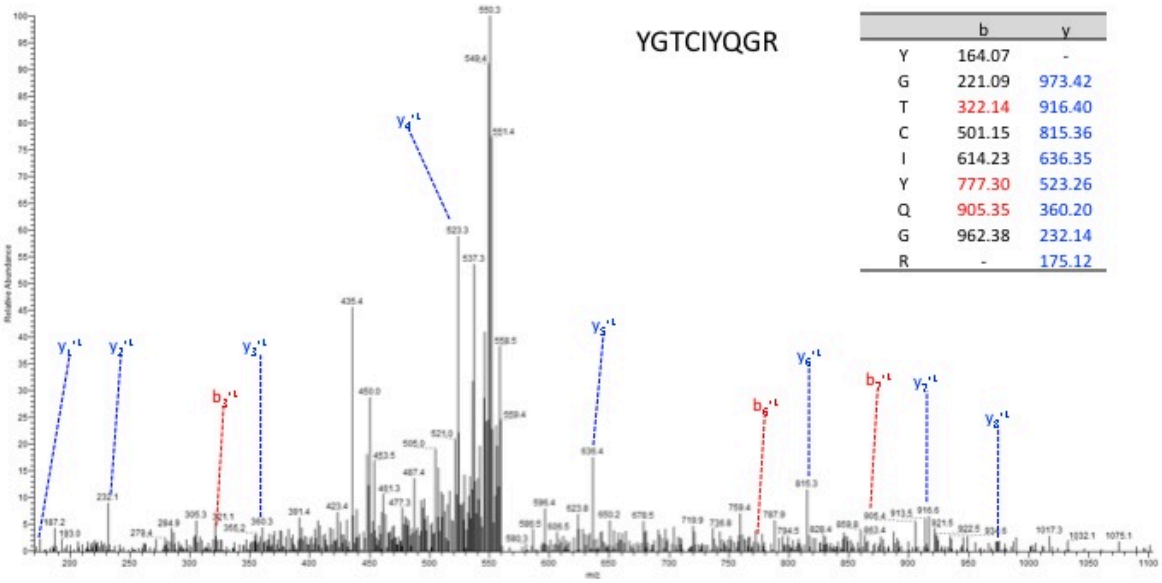

Fig S2. Identification of HNP in immune complexes of SLE patients. HPLC-ESI/MS/MS analysis of small peptides (size between 4 and 6 kD) contained in purified immune complexes detected an HNP tryptic peptide with a mass-to-charge ratio of 568.25 at the retention time of 15.82 min. A typical MS/MS spectrum of YGTCIYQGR peptide of HNP is shown.

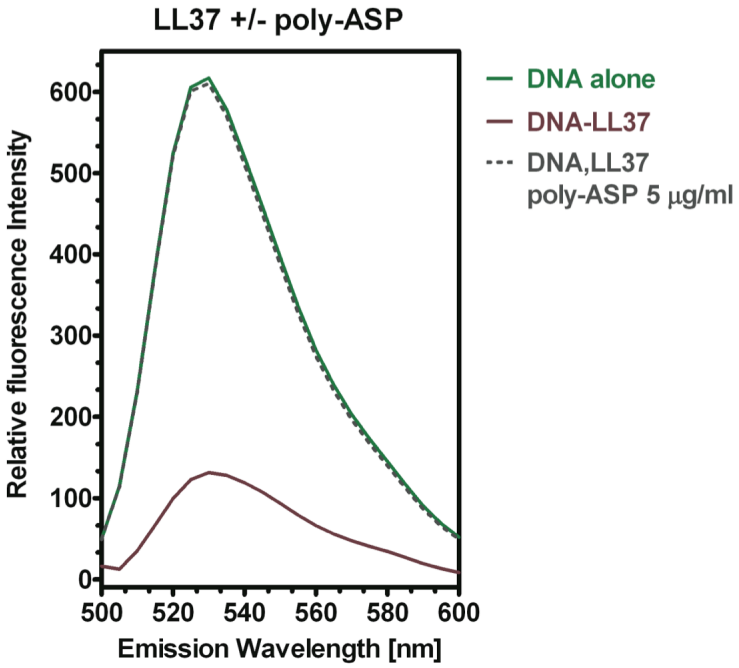

Fig S3. Polyanionic polymers reverse the complex formation between cationic antimicrobial peptides and DNA. Fluorescence of human DNA (3 μg ml^{-1}) stained with PicoGreen either alone or in complex with LL37 (10 μM). The decrease in DNA fluorescence is indicative of binding of the peptides to DNA and results from both dye exclusion as well as quenching of the fluorescence. Treatment of the complexes with an excess of negative charges using polyaspartic acid (poly-ASP, 5 μg ml^{-1}) resulted in detachment of the DNA from the peptide, as shown by the fact that the dye acquired the ability to fully stain the DNA. One representative of at least 3 independent experiments is shown.

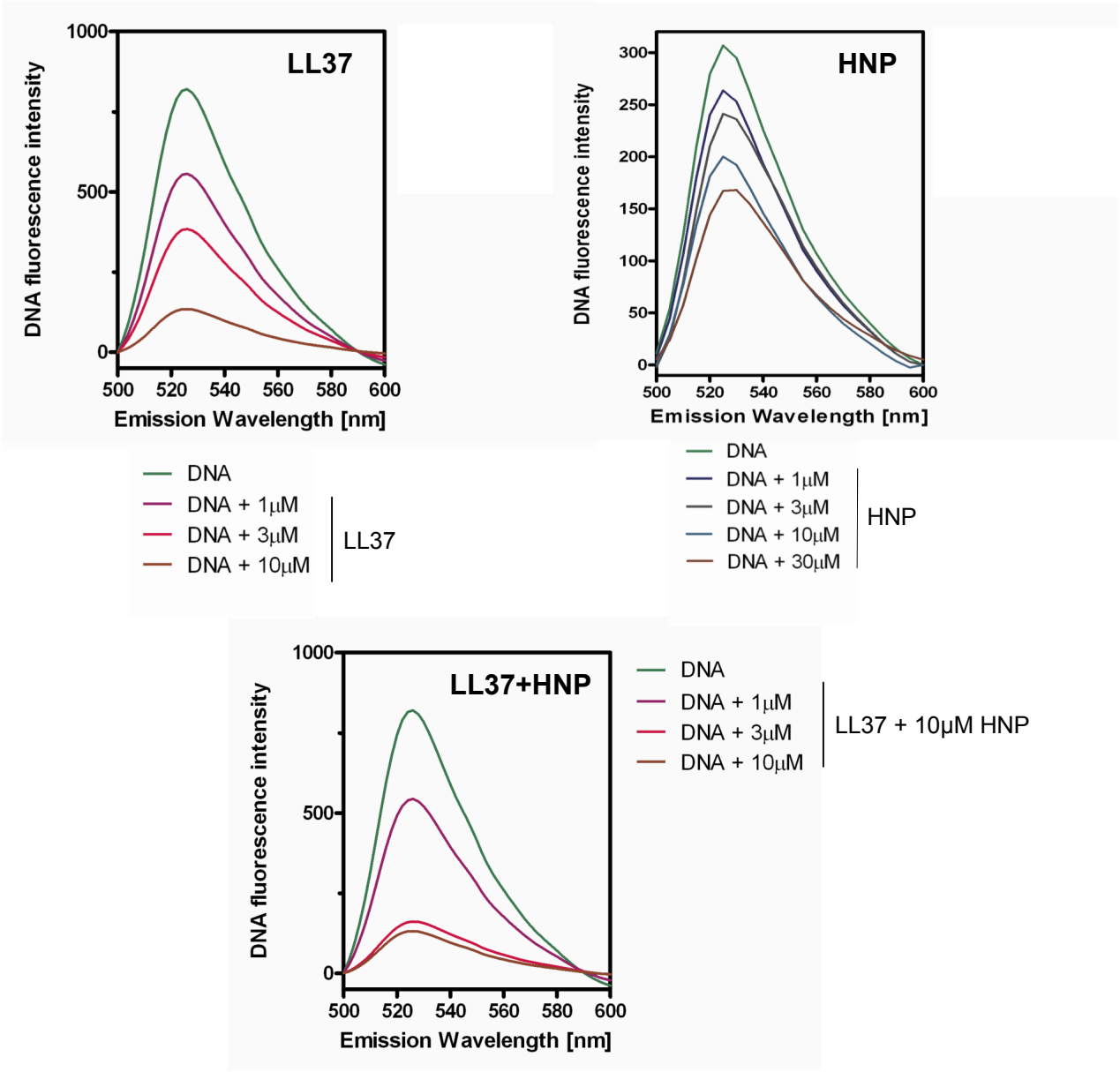

Fig. S4. LL37 and HNP both bind human DNA but only LL37 induces its condensation. Fluorescence of DNA stained with PicoGreen dye either alone or in complex with increasing concentrations of LL37 (*upper panel*) or human neutrophil peptide, isoforms 1-3 [$HNP_{(1-3)}$] (*middle panel*). Both LL37 and HNP can form a complex with DNA as shown by the moderate, gradual decrease in DNA fluorescence, but only LL37 induced DNA condensation, as shown by the strong and sudden decrease in DNA staining occurring at 10 μM LL37. DNA condensation was associated with the formation of insoluble particles, the protection from DNase-induced degradation, and the ability of the DNA to trigger plasmacytoid dendritic cell (pDC) activation. Fluorescence of DNA stained with PicoGreen in complex with increasing concentrations of LL37 alone or in the presence of 10 μM $HNP_{(1-3)}$ (*lower panel*) shows that HNPs promote DNA condensation in the presence of suboptimal LL37 concentrations (3 μM). One of at least 3 independent experiments is shown.

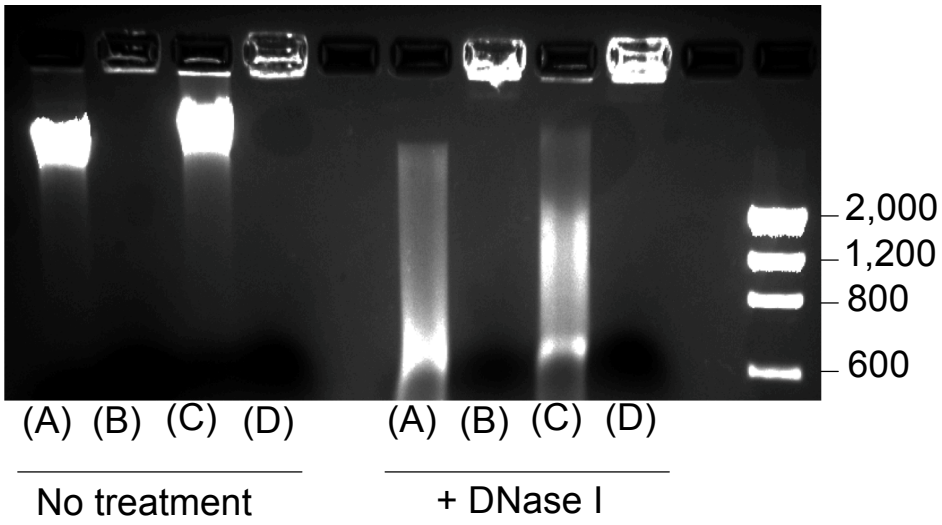

Fig. S5. LL37-containing DNA complexes are protected from enzymatic degradation. Agarose gel electrophoresis of human DNA alone (A), human DNA in complex with LL37 (B), anti-DNA antibodies (C), or LL37 plus anti-DNA antibodies (D) before and after treatment with DNase I. The DNA was visualizedby ethidium bromide staining. LL37-containing DNA complexes were retained in the loading wells due to size and/or charge neutralization and were protected from DNase-induced degradation.

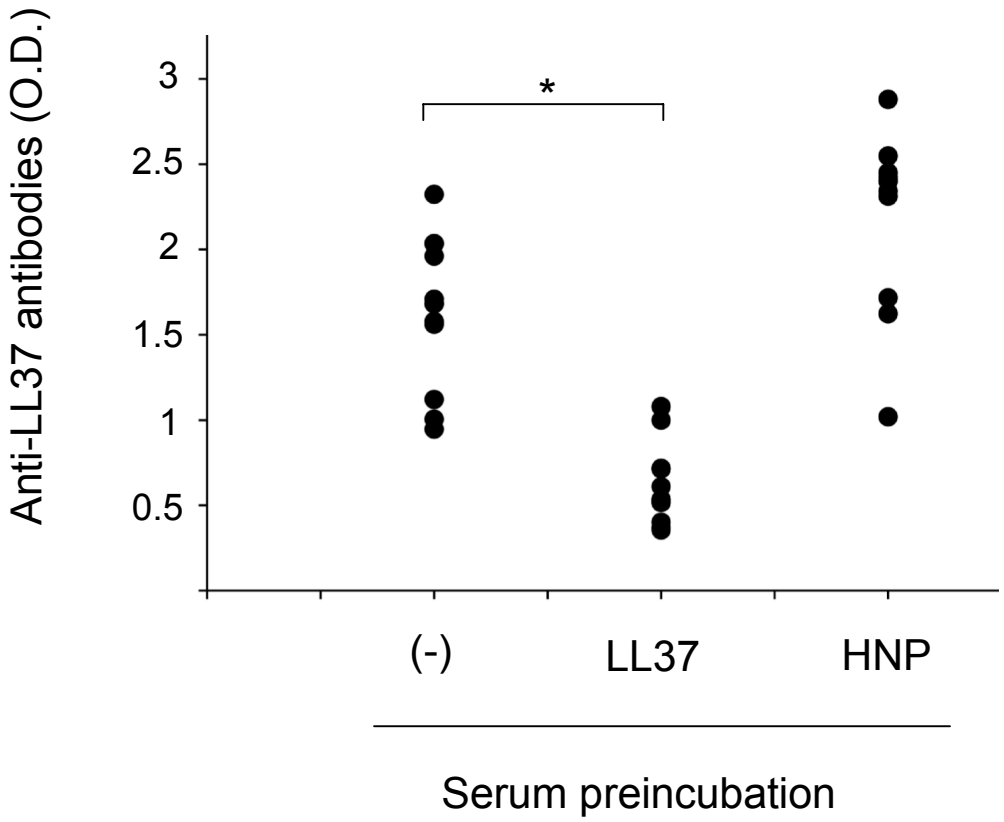

Fig. S6. Specific blocking of IgG reactivity to LL37. Sera of 10 SLE having high levels of anti-LL37 antibodies were preincubated with saturating concentrations of LL37 peptide or HNP before reactivity to plate bound LL37 was determined by ELISA. Results are expressed as optical density (OD) and show that reactivity to LL37 can be blocked by preincubation of the sera with LL37 but not HNP peptides. *, $p < 0.0001$; Student's t test.

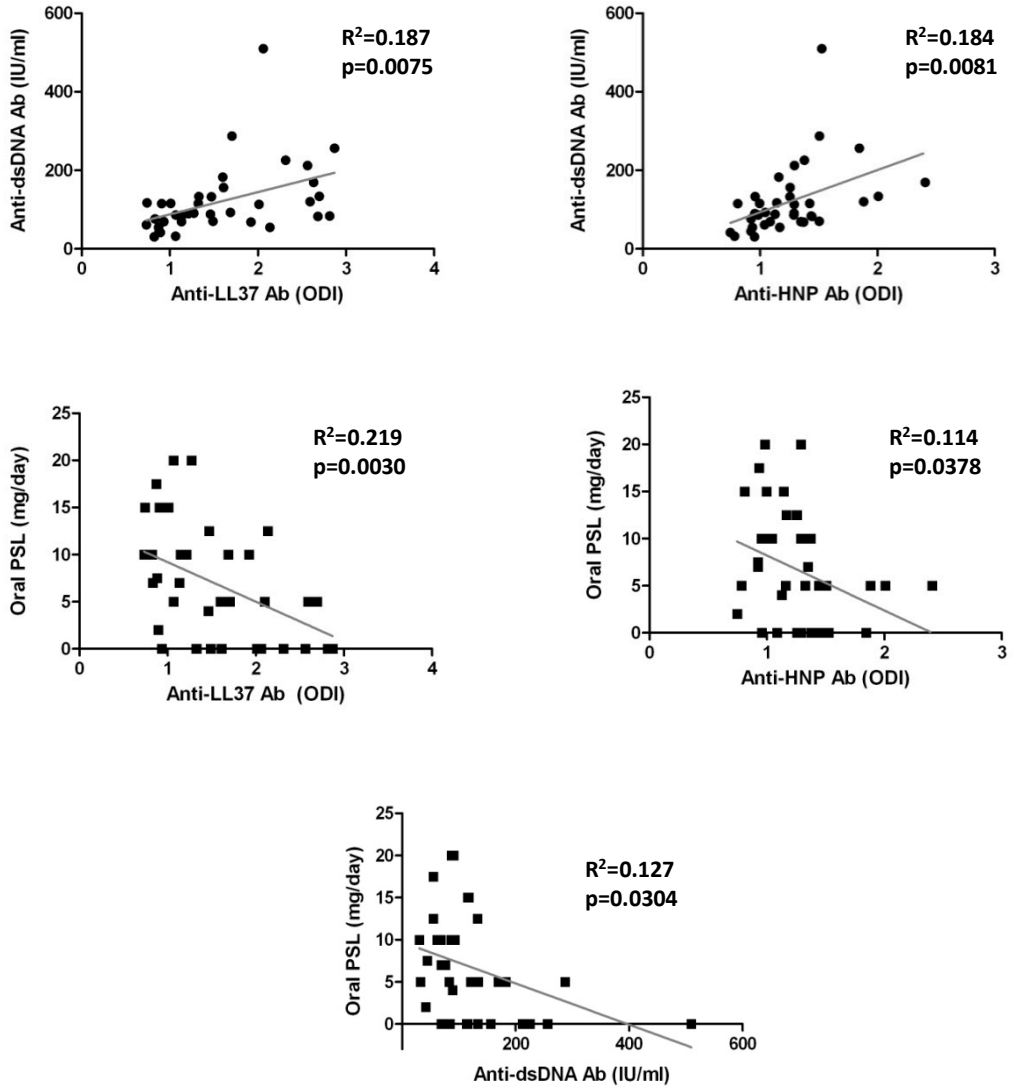

Fig. S7. Correlations of antibodies against antimicrobial peptides with levels of anti-DNA antibodies and dose of prednisolone. Levels of antibodies to LL37 and HNP were correlated with the anti-DNA antibody levels and the oral dose of prednisolone (PSL) in 38 SLE patients. Two-tailed Pearson's correlation test was used, correlation coefficient (R^2) and significance (p) are given in the figures.

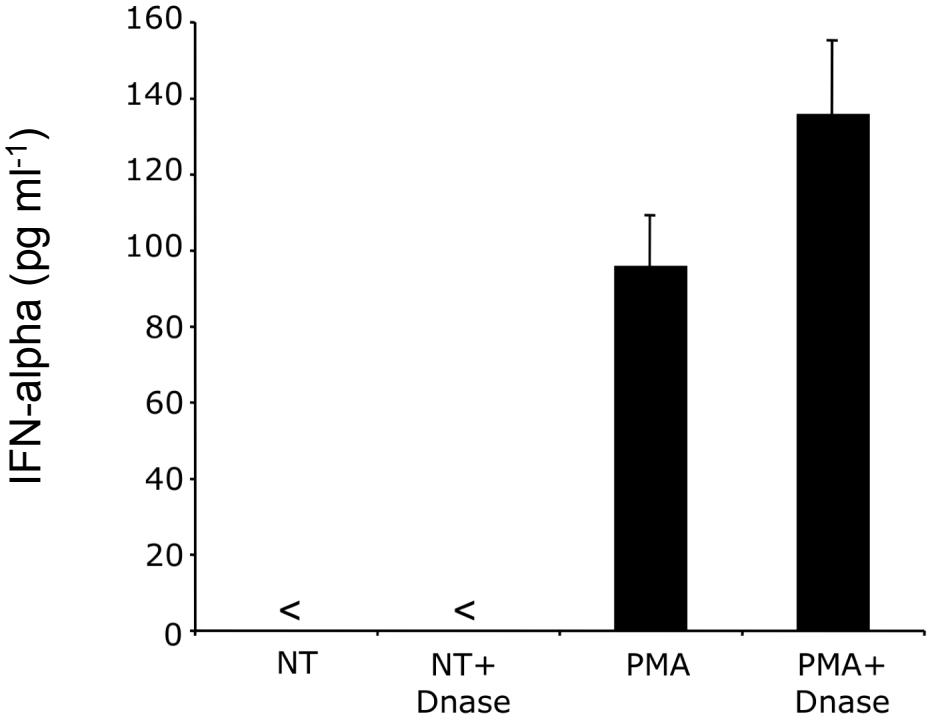

Fig. S8. Treatment of NETs with DNAse I does not abrogate pDC activation. Supernatant of untreated neutrophils (NT) or neutrophils activated for 3h with PMA to induce NETs were treated for 30min with DNase I (10U/ml) and used to stimulate pDC. The DNase treatment was shown to deplete the long DNA stretches but not the globular DNA-antimicrobial peptide domains in NETs. IFN-α was measured in the supernatants after overnight culture of pDCs. <, below lowest standard of 12.5 pg ml^{-1}. Data represent the mean plus SD of triplicate wells.

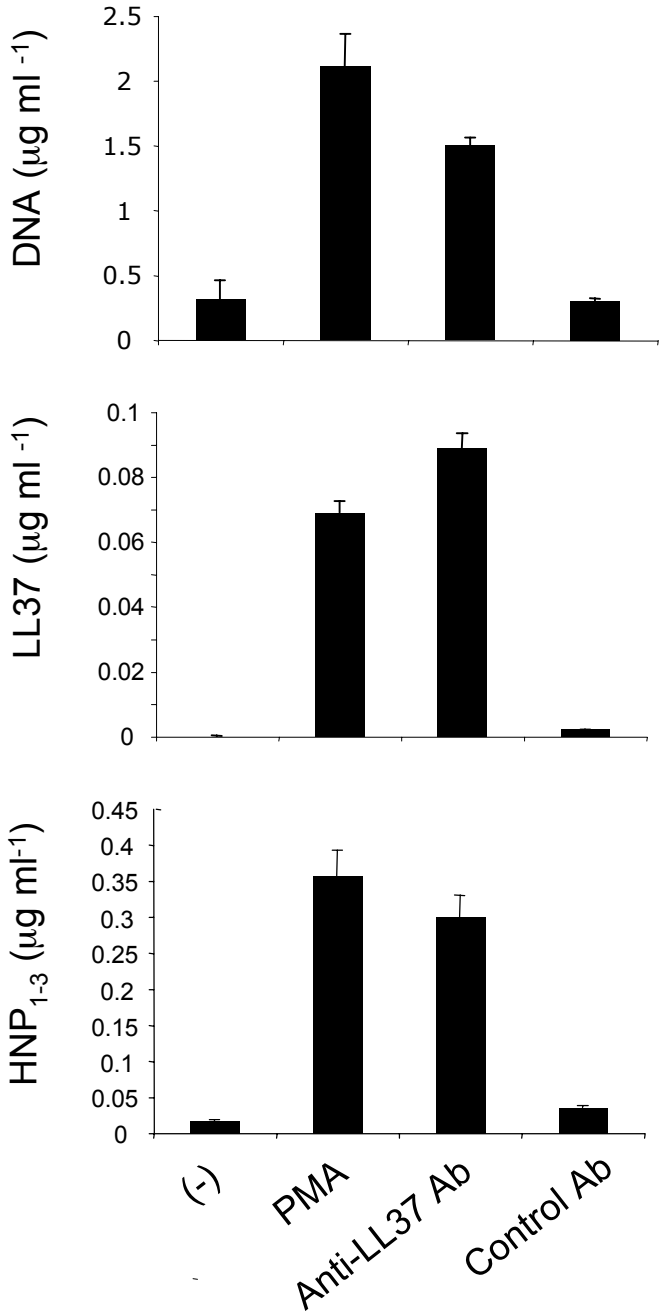

Fig. S9. Quantification of DNA, LL37, and HNPs in the supernatants of netting neutrophils. Purified neutrophils were cultured for 3h either unstimulated, or stimulated with PMA, anti-LL37 antibodies or a control antibody before the supernatants were collected. Contents of DNA were measured using the picogreen assay, LL37 and HNPs were measured using commercially available ELISA kits. Data represent the mean plus SD of two independent experiments.

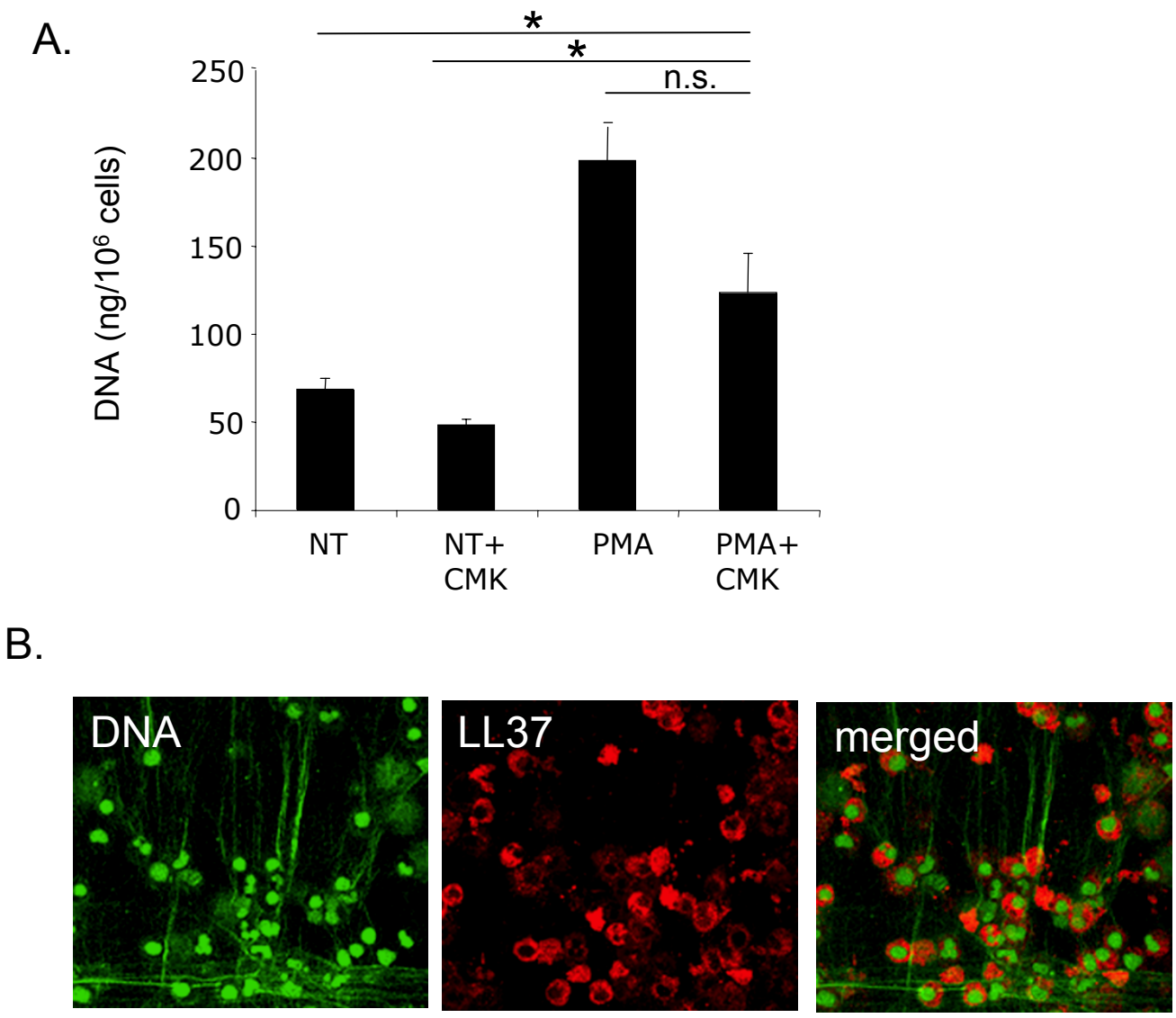

Fig. S10. NET release by neutrophils treated with CMK to block the cleavage of LL37 from hCAP18. A. Purified neutrophils were either left untreated or pretreated with CMK before activation with PMA. After 3h, DNA content () was measured in cell-free supernatants using the picogreen assay. Data represent the mean plus SD of four independent experiments. ns, not significant, *, $p < 0.035$; Student's t test. **B.** Confocal microscopy of NETs released by neutrophils pretreated with CMK and activated with PMA before staining for DNA and LL37.

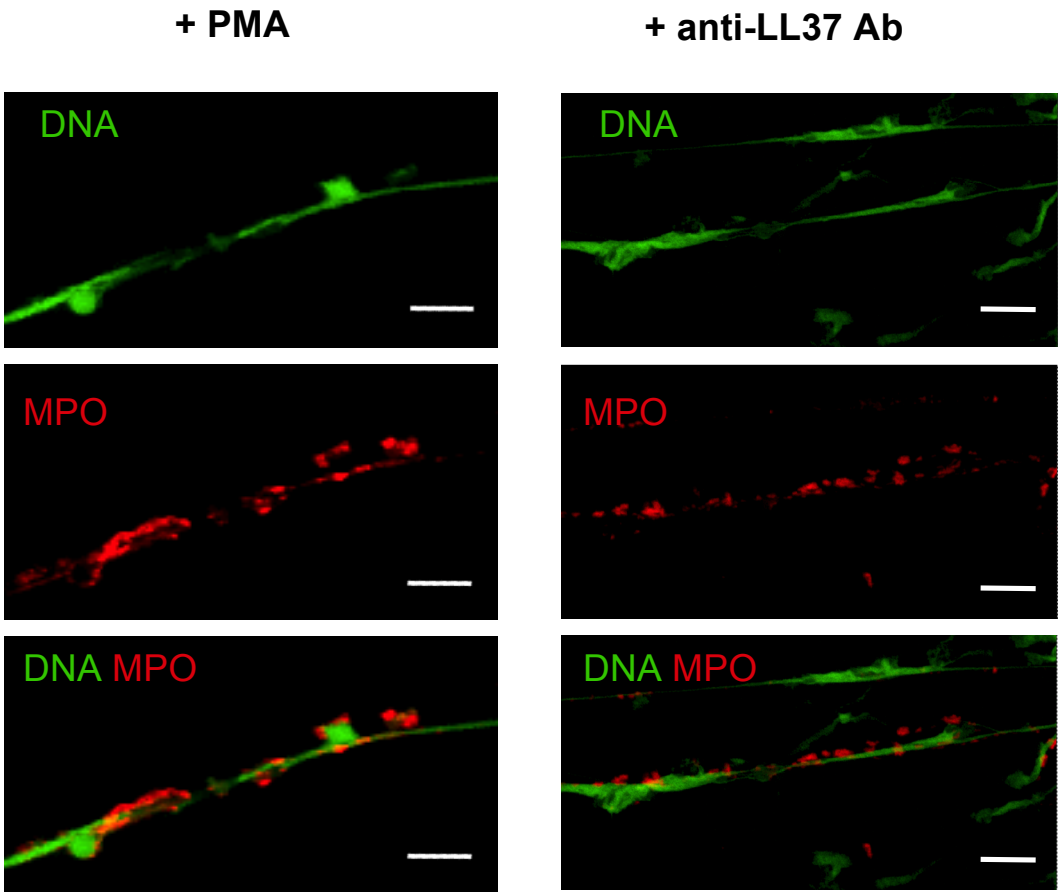

Fig. S11. Visualization of NETs. Neutrophils were activated with phorbol myristate acetate (PMA) or anti-LL37 antibodies to induce NETs. NETs were stained with a DNA dye (DAPI, shown in green) and an antibody against myeloperoxidase MPO (from Abcam) followed by Alexa546-labeled goat anti mouse IgG (from Invitrogen). A representative image is shown. Bars = 10 μm.

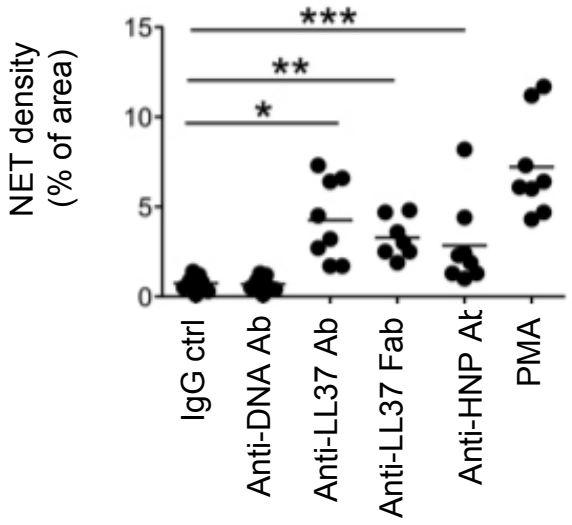

Fig. S12. Automated quantification of NETosis by neutrophils activated with different stimuli. An automated density score was obtained by analyzing the images used for Fig. 6A with the imageJ software (public software developed by NIH). After applying a fluorescence threshold, this software measures the percentage of pixels with fuorescence among the total pixels of the image. *, p = 0.0207; **, p = 0.002; ***, p = 0.0066; ANOVA (adjusted for Dunnett's test).

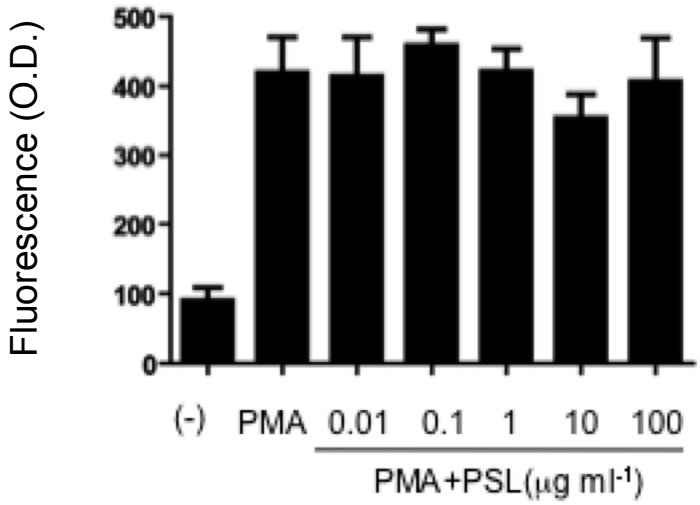

Fig. S13. Corticosteroids do not affect the ability of neutrophils to release NETs. Purified neutrophils were left untreated or pretreated for 1h with increasing concentrations of methylprednisolone (PSL, from APP Pharmaceuticals, Schaumburg, IL), followed by a 3h activation with PMA. NET-DNA was quantified in the supernatants using the Picogreen assay. Data are given as fluorescence O.D. of one representative out of two experiments. Mean plus SD of duplicate wells are shown.

SLE patients	Sex	Age	Anti-DNA Ab (IU/ml)	Anti-LL37 Ab (ODI)	Anti-HNP Ab (ODI)	Total IgG (g/ml)	Disease activity	Proteinuria	Serum IFN-α (pg/ml)	Oral PSL (mg/day)
1	F	54	182.8 **(+)**	1.6	1.16 **(+)**	12.08	(-)	(-)	0	5
2	F	35	86.4	1.067	0.984	9.71	**(++)**	(+)	24	20
3	F	30	89.6	1.212	0.955	5.44	**(+)**	(-)	0	10
4	M	58	69.1	0.933	1.086	7.29	**(++)**	n.d.	0	0
5	F	17	256.2 **(+)**	2.87 **(+)**	1.845 **(+)**	17.34	**(+++)**	(+)	50	0
6	F	22	115.6	1.011	0.995	5.72	**(+)**	(+)	0	15
7	F	48	117.3	0.741	1.142	9.26	**(+)**	(+)	0	15
8	F	36	76.1	0.832	0.922	7.52	**(++)**	(+)	0	7
9	F	48	54.9	0.873	0.934	7.29	(-)	(-)	0	17.5
10	F	39	61.4	0.734	1.037	5.37	**(++)**	(+)	0	10
11	F	52	44.6	0.879	0.922	8.37	(-)	(-)	0	7.5
12	F	22	115.1	0.908	0.809	8.07	**(+)**	(+)	0	15
13	F	39	30.6	0.823	0.952	8.19	**(+)**	(+)	0	10
14	F	42	32.1	1.065	0.782	4.75	(-)	(-)	0	5
15	F	44	88.4	1.46	1.128	5.61	(-)	(-)	0	4
16	F	56	287.1 **(+)**	1.705 **(+)**	1.506 **(+)**	9.47	**(+)**	(-)	0	5
17	F	25	70.3	1.491	1.505 **(+)**	13.18	(-)	(-)	0	0
18	F	37	69	1.133	1.348 **(+)**	8.76	(-)	(-)	0	7
19	F	46	133.4 **(+)**	1.33	0.957	12.42	**(+)**	(+)	0	0
20	M	58	41.5	0.892	0.746	4.52	(-)	(-)	0	2
21	F	48	90.5	1.272	1.289 **(+)**	10.43	**(+)**	(+)	0	20
22	F	15	83.9	2.814 **(+)**	1.436 **(+)**	7.96	**(+)**	(-)	7.165	0
23	F	57	113	2.013 **(+)**	1.291 **(+)**	8.47	(-)	(-)	0	0
24	F	26	n.d.	2.098 **(+)**	1.324 **(+)**	12.04	(-)	(-)	0	5
25	F	35	212.2 **(+)**	2.564 **(+)**	1.292 **(+)**	17.42	**(+)**	(-)	56.03	0
26	F	52	133.8 **(+)**	2.696 **(+)**	2.008 **(+)**	14.31	**(+)**	(+)	15.488	5
27	F	58	82.6	2.68 **(+)**	1.441 **(+)**	3.25	**(+)**	(-)	0	5
28	F	50	509.6 **(+)**	2.062 **(+)**	1.526 **(+)**	13.51	(-)	(-)	0	0
29	M	51	115.6	1.323	1.422 **(+)**	7.51	**(++)**	(+)	0	0
30	F	48	169.2 **(+)**	2.633 **(+)**	2.408 **(+)**	12.42	**(+)**	(+)	0	5
31	F	26	86.1	1.145	1.287 **(+)**	8.42	**(+)**	(+)	0	10
32	F	52	120.4 **(+)**	2.593 **(+)**	1.883 **(+)**	30.49	**(+)**	(+)	13.877	5
33	F	41	225.6 **(+)**	2.314 **(+)**	1.378 **(+)**	16.52	**(+)**	(-)	0	0
34	F	28	68.1	1.921 **(+)**	1.371 **(+)**	9.3	**(++)**	(-)	59.521	10
35	F	29	156.1 **(+)**	1.611 **(+)**	1.256 **(+)**	12.79	**(+)**	(-)	19.516	0
36	F	46	92.5	1.687 **(+)**	1.045	8.42	**(+)**	(-)	0	10
37	F	38	132.9 **(+)**	1.473	1.254 **(+)**	7.12	**(+)**	(-)	0	12.5
38	F	56	54.8	2.136 **(+)**	1.168 **(+)**	4.58	**(+)**	(-)	13.609	12.5

Table S1: Characteristics of SLE patients

Anti-DNA Ab: (+) > 115 IU/ml.
Anti-LL37 Ab: (+) > 1.611 ODI.
Anti-HNP Ab: (+) > 1.15 ODI.
Disease activity: (-) SLEDAI<6, (+) SLEDAI 6-12, (++) SLEDAI 12-20, (+++) SLEDAI>20.
Proteinuria: (-) absent, (+) present.
PSL: prednisolone.

Scleroderma	Sex	Age	Type	Ulcers	PF	PAH	CAP	Ab	Anti-DNA Ab (IU/ml)	Anti-LL37 Ab (ODI)	Anti-HNP Ab (ODI)	Total IgG (g/ml)
1	F	38	D	YES	YES	NO	LATE	SCL70	116.49	1.008	1.011	8.12
2	F	62	L	NO	NO	NO	ACTIVE	CENT	58.37	0.43	0.382	3.96
3	F	63	L	NO	YES	NO	ACTIVE	SCL70	34.84	0.69	0.585	5.39
4	M	68	L	NO	NO	YES	ACTIVE	CENT	26.58	0.719	0.572	5.77
5	M	68	L	YES	NO	YES	ACTIVE	CENT	47.64	0.66	0.703	4.21
6	F	41	D	YES	YES	NO	LATE	SCL70	134.99 (+)	0.669	0.58	9.13
7	F	63	D	YES	YES	NO	LATE	ANA	58.7	0.573	0.681	3.71
8	F	62	L	NO	YES	NO	LATE	SCL70	34.51	0.46	0.451	7.36
9	F	62	D	YES	YES	NO	ACTIVE	SCL70	71.58	0.3343	0.315	4.11
10	F	73	L	NO	NO	NO	EARLY	CENT	85.37	0.9014	0.791	7.88
11	F	42	D	YES	YES	NO	LATE	SCL70	81.82	0.531	0.497	4.84
12	F	63	D	YES	YES	NO	EARLY	SCL70	146.55 (+)	0.988	0.886	8.27
13	F	62	D	YES	YES	NO	LATE	SCL70	63.32	1.738	1.502 **(+)**	10.92
14	F	58	D	YES	YES	NO	LATE	SCL70	34.59	0.826	0.694	4.76
15	F	50	D	YES	YES	NO	ACTIVE	ANA	77.36	1.826	1.539 **(+)**	12.12
16	F	56	D	YES	YES	NO	LATE	SCL70	100.32	1.382	1.252 **(+)**	5.21
17	F	65	L	YES	NO	YES	LATE	CENT	71.99	1.079	1.063	5.91
18	F	51	L	NO	NO	NO	EARLY	CENT	36.57	0.62	0.572	5.46
19	F	61	L	YES	NO	YES	ACTIVE	CENT	37.73	1.138	1.127	6.22
20	F	68	L	YES	YES	YES	ACTIVE	SCL70	142.26 (+)	1.047	1.036	1.21
21	F	75	L	NO	NO	YES	ACTIVE	CENT	145.73 (+)	1.493	1.5	21.22
22	F	45	D	NO	NO	NO	EARLY	SCL70	74.3112	1.179	1.152	5.87
23	F	63	L	YES	NO	YES	LATE	CENT	128.47	1.567	1.278 **(+)**	10.34
24	F	73	L	YES	YES	NO	LATE	CENT	86.69	0.467	0.51	5.34
25	F	64	D	YES	YES	NO	LATE	SCL70	56.39	0.668	0.614	5.52
26	F	74	L	NO	YES	NO	ACTIVE	ANA	91.07	0.911	0.4728	9.03
27	F	50	L	NO	YES	NO	NORMAL	SCL70	67.62	0.44	0.392	6.43
28	F	42	D	YES	YES	NO	LATE	SCL70	74.55	0.818	0.498	7.75
29	F	55	L	NO	NO	NO	EARLY	NEG	56.97	0.374	0.33	1.85
30	F	28	L	NO	NO	NO	ACTIVE	SCL70	56.31	0.763	0.637	5.74

Table S2. Characteristics of scleroderma patients.
Type: D=Diffuse, L=Localized.
PF= Pulmonary Fibrosis.
PAH= Pulmonary Arterial Hypertension.
CAP: scleroderma classified into “early”, “active” and “late” according to nailfold capillary abnormalities
Ab: characteristic autoantibodies

Healthy	Sex	Age	Anti-DNA Ab (IU/ml)	Anti-LL37 Ab (ODI)	Anti-HNP Ab (ODI)	Total IgG (g/ml)
1	F	38	110.14	1.39	1.08	8.12
2	F	62	103.54	1.1	1.64 **(+)**	3.96
3	F	63	97.67	1.13	0.95	5.39
4	F	33	116.48	1.13	0.94	5.77
5	M	31	96.19	0.92	0.91	4.21
6	M	31	76.87	0.96	1.08	9.13
7	M	33	76.87	1.04	0.74	3.71
8	F	29	110.54	0.84	0.99	7.36
9	F	26	93.96	0.91	1.04	4.11
10	F	33	n.d	0.81	0.9	7.88
11	F	31	n.d	0.89	1.0	4.84
12	F	31	n.d	0.84	0.67	8.27

Table S3. Characteristics of healthy control donors.

		Anti-LL37	Anti-HNP	Anti-dsDNA	Total IgG	Serum IFN-α	Disease Activity	Proteinuria
Anti-LL37	Correlation (R^2)	1,000	**0.592**	**0.187**	**0.272**	**0.194**	0.009	0.033
	P (2-tailed)	-	**<0.0001**	**0.0075**	**0.0008**	**0.0057**	0.5735	0.2806
Anti-HNP	Correlation (R^2)		1,000	**0.184**	**0.333**	0.065	0.028	0.025
	P (2-tailed)		-	**0.0081**	**0.0001**	0.1225	0.3182	0.3505
Anti-dsDNA	Correlation (R^2)			1,000	**0.188**	0.023	<0.001	0.006
	P (2-tailed)			-	**0.0074**	0.3726	0.9518	0.6449
Total IgG	Correlation (R^2)				1,000	**0.142**	0.008	0.04
	P (2-tailed)				-	**0.0195**	0.5896	0.235
Serum IFN-α	Correlation (R^2)					1,000	**0.221**	<0.001
	P (2-tailed)					-	**0.0029**	0.9716

Table S4. Correlation between parameters of SLE patients. Two-tailed Pearson's correlation test was used, correlation coefficient (R2) and significance (p) are given.

REFERENCE

1. Jung, S.Y. *et al.* Complications in the assignment of 14 and 28 Da mass shift detected by mass spectrometry as in vivo methylation from endogenous proteins. *Analytical Chemistry* 80, 1721-1729 (2008)