Table ST1. Virtual colony count antibacterial activities of pro-cathelicidin and LL-37

	Virtual lethal doses (μM)			
	vLD_{50}	vLD_{90}	vLD_{99}	$vLD_{99.9}$
Escherichia coli ATCC 25922				
HNP1[1]	0.98 ± 0.11	2.17 ± 0.27	15.71 ± 10.91	26.31 ± 15.43
LL-37	0.67 ± 0.08	1.30 ± 0.09	Footnote 2	Footnote 3
hCLD	> 21	> 21	> 21	> 21
proLL-37 16-0.06	1.02 ± 0.02	1.27 ± 0.04	1.75 ± 0.11	2.69 ± 0.09
proLL-37 8-0.03	1.00 ± 0.03	1.30 ± 0.07	1.84 ± 0.16	2.69 ± 0.14
Enterobacter aerogenes ATCC 13048				
HNP1	2.36 ± 0.09	10.35 ± 1.61	Footnote 4	> 74
LL-37	0.71 ± 0.11	1.29 ± 0.04	Footnote 5	Footnote 6
hCLD	> 21	> 21	> 21	> 21
proLL-37 16-0.06	0.98 ± 0.13	1.43 ± 0.15	2.19 ± 0.29	3.26 ± 0.44
proLL-37 8-0.03	1.22 ± 0.12	1.71 ± 0.30	2.22 ± 0.29	2.84 ± 0.29
Staphylococcus aureus ATCC 29213				
HNP1	3.15 ± 1.11	Footnote 7	Footnote 8	Footnote 9
LL-37	1.24 ± 0.15	1.75 ± 0.15	2.27 ± 0.11	2.87 ± 0.15
hCLD	> 21	> 21	> 21	> 21
proLL-37 16-0.06	11.99	> 16	> 16	> 16
proLL-37 8-0.03	> 8	> 8	> 8	> 8

The virtual LD_{50} (vLD_{50}), vLD_{90}, vLD_{99} and $vLD_{99.9}$ were reported as the tested protein concentrations that resulted in survival rates of 0.5, 0.1, 0.01 and 0.001, respectively. The values represent an average of three independent experiments, except as follows: against *E. coli* hCLD and the proLL-37 series between 8 and 0.03 μM are in duplicate, and at 3.6 μM LL-37 is reported in duplicate, because its threshold time was >720 minutes in the third replicate; against *E. aerogenes* hCLD is in duplicate, the proLL-37 series between 8 and 0.03 μM is in duplicate except for 8 μM, which is a single data point because the other replicate had a threshold time of >720 minutes, in the proLL-37 series between 16 and 0.06 μM, the 16 μM is in duplicate because the threshold time of the third replicate was >720 minutes, and at 3.6 μM LL-37 is reported in duplicate, because its threshold time was >720 minutes in the third replicate; against *S. aureus* hCLD and the proLL-37 series between 8 and 0.03 μM are in duplicate, and in two cases single results are reported because at the 7 and 14 μM concentrations of LL-37, two assays resulted in threshold times of > 720 minutes and the third did not.

[1]human α-defensin 1 (HNP1) was used as positive control

[2]The three vLD_{99} values were $1.8 < vLD_{99} < 3.6$, 1.96, and 1.95

[3]The three $vLD_{99.9}$ values were $1.8 < vLD_{99.9} < 3.6$, 2.25, and 2.27.

[4]The three vLD_{99} values were 30.13, >74, and 55.68

[5]The three vLD_{99} values were 2.26, 1.87, and $0.9 < vLD_{99} < 1.7$.

[6]The three $vLD_{99.9}$ values were 3.14, 2.31, and $0.9 < vLD_{99.9} < 1.7$.

[7]The three vLD_{90} values were 9.14, 7.63 and $9.3 < vLD_{90} < 18.6$.

[8]The three vLD_{99} values were $18.6 < vLD_{99} < 37.2$, 10.89, and $9.3 < vLD_{99} < 18.6$.

[9]The three $vLD_{99.9}$ values were $18.6 < vLD_{99.9} < 37.2$, 13.18, and $9.3 < vLD_{99.9} < 18.6$.

Table ST2. Colony count antibacterial activities of HNP1, LL-37, hCLD and pro-cathelicidin.

	LD (mM)	
	LD_{50}	LD_{90}
E. coli ATCC 25922		
HNP1	0.12 ± 0.09	> 29
LL-37	0.21 ± 0.16	$0.6 < LD_{90} < 4$
hCLD	> 8	> 8
pro-cathelicidin	0.52 ± 0.28	$1 < LD_{90} < 6$
S. aureus ATCC 29213		
HNP1	19.0 ± 3.8	> 29
LL-37	$0.6 < LD_{50} < 4$	$0.6 < LD_{90} < 4$
hCLD	> 8	> 8
pro-cathelicidin	> 6	> 6

The LD_{50} and LD_{90} were calculated as the tested protein concentration that resulted in survival rates of 0.5, and 0.1 respectively in the colony count assay. The values represent an average of two independent experiments. HNP1 was weaker in the colony count assay than the vCC assay at high concentrations, in accordance with the fact that vCC reports lag times in addition to bacteriostatic activity and bactericidal killing (*1*) and initial reports of a lack of HNP activity at 50 µg/mL in unsupplemented 10 mM sodium phosphate buffer (*2*). Differences between colony count LD_{50} and vCC vLD_{50} could reflect an inoculum effect, since the concentration of cells exposed to peptides was 20-fold higher for vCC, and differences could also reflect that nutrients carried forward from the initial seed culture were 20-fold more dilute in the colony count assay.

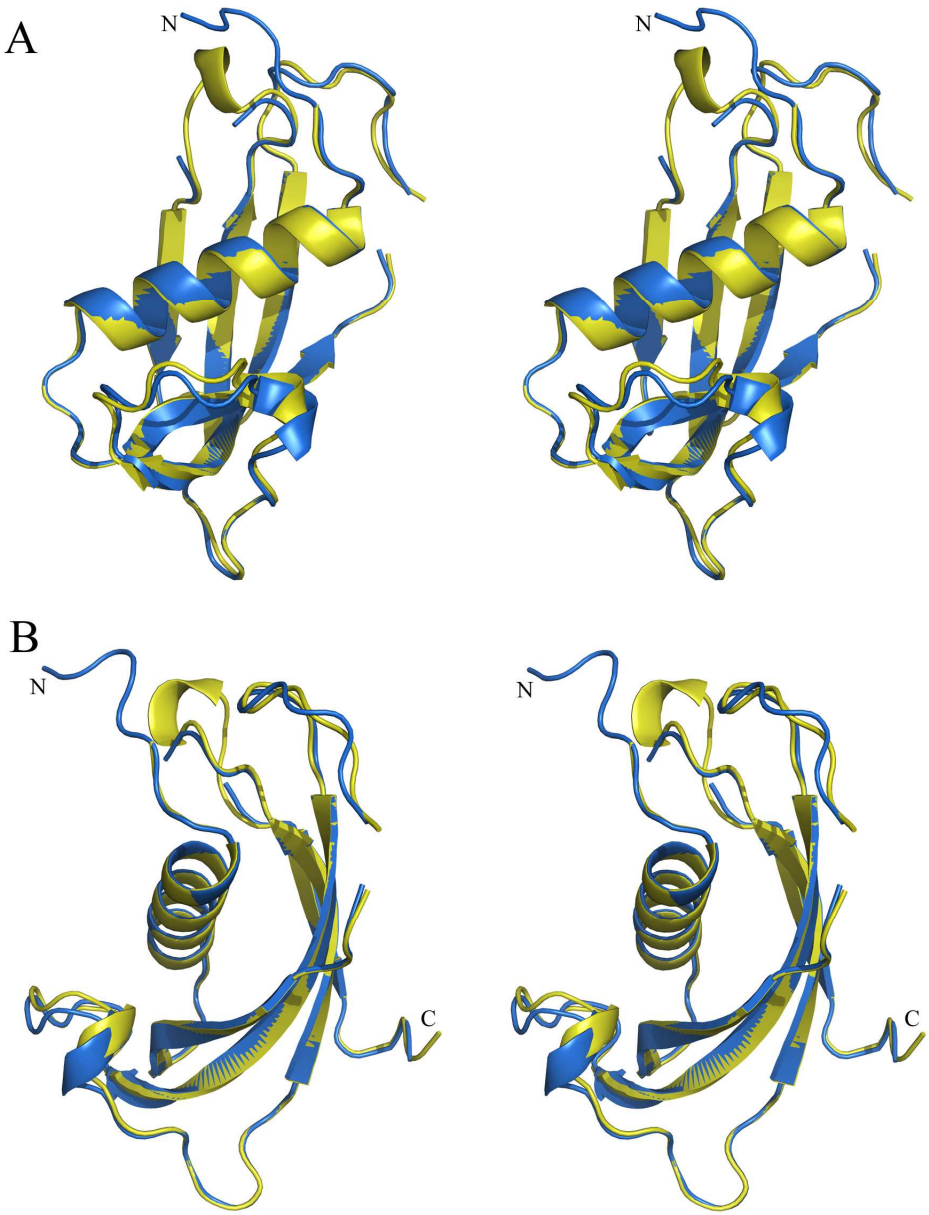

Figure S1. Stereo view of a superposition (A) and its 90° rotation (B) of hCLD molecules present in the asymmetric unit of the crystal**.** Structures can be superimposed with the root mean square deviation of equivalent Cα atoms of 0.61 Å.

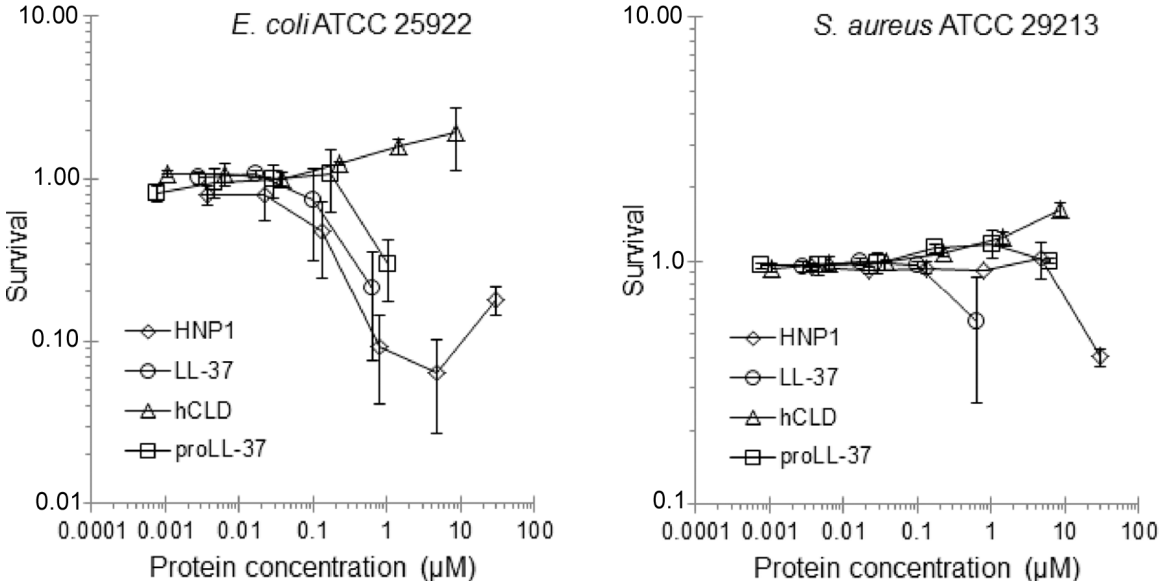

Figure S2. Antibacterial activity of pro-cathelicidin, hCLD and LL-37 determined by the traditional colony count method**.** Survival curves are shown of (A) *E. coli* ATCC 25922 and (B) *S. aureus* ATCC 29213 exposed to protein concentrations varying six-fold from 0.013 to 100 µg/mL in 10 mM sodium phosphate buffer, and converted to µM for plotting. Costar 3595 96-well plates were used for the 2-hour defensin exposure at 37 ºC and subsequent 50-to-100-fold dilutions in buffer before spreading on Meuller Hinton agar plates and incubating overnight at 30-37 ºC. 2-3 mm diameter colonies were counted and plotted with the standard error of the mean for duplicate experiments. HNP1 was used as positive control. Points scored as zero survival, 16.7 and 100 µg/mL (3.7 and 22.3 µM) LL-37 against both *E. coli* and *S. aureus* and 100 µg/mL (6.1 µM) proLL-37 against *E. coli*, could not be plotted.

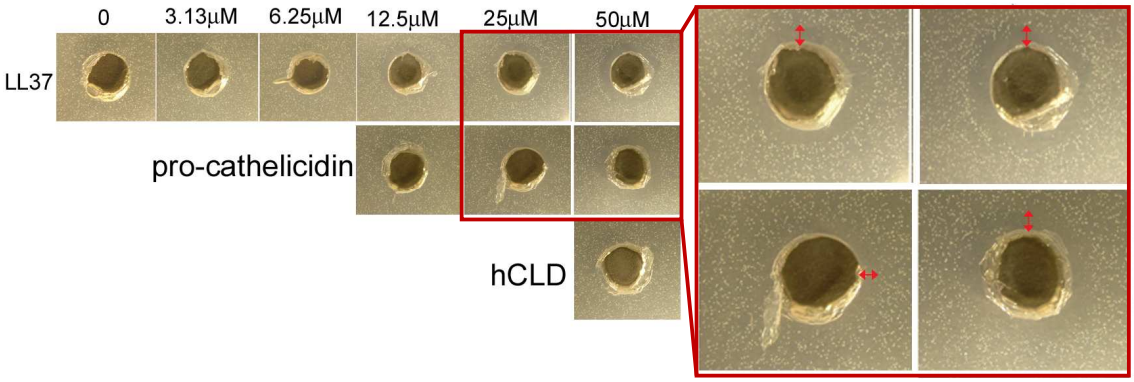

Figure S3. Anti-*E.coli* 25922 activity of LL-37, pro-cathelicidin and hCLD as measured using a standard radial diffusion assay (*3*). Clear areas on tryptone/agarose plates indicate antibacterial activity causing a decrease in bacterial colony density. The highest two concentrations of LL-37 and pro-cathelicidin are enlarged with red bars showing the relative diameters. LL37 shows antimicrobial activity at 50uM, 25uM and 12.5uM and pro-cathelicidin at two highest concentrations tested of 50uM and 25 uM. hCLD shows no antibacterial activity. The radial diffusion assay was conducted in a different laboratory than the virtual colony count assay.

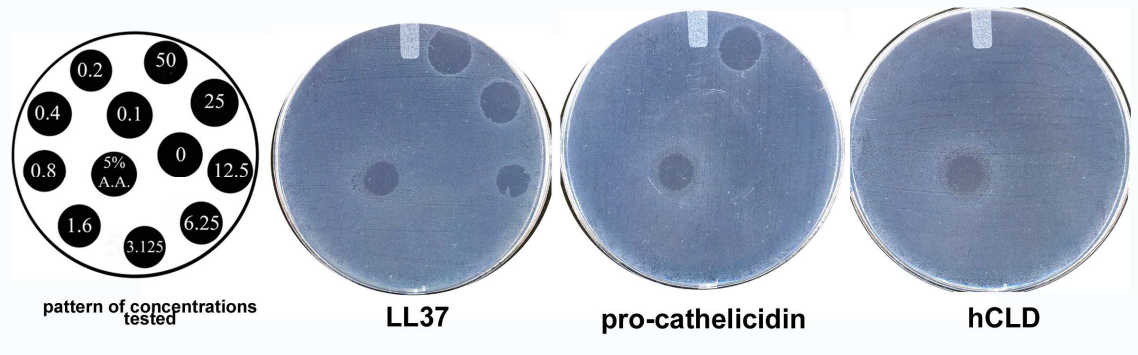

Figure S4. Anti-*E.coli* activity of LL-37, pro-cathelicidin and hCLD as measured using a lawn-spotting assay (*4*). 10ul of serial solutions (0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50uM) of each peptide were spotted on the surface of the bacterial lawn. 5% Acetic Acid was used as a positive control. Clear spotted areas indicate antibacterial activity causing a decrease in bacterial colony density. LL37 show obvious antimicrobial activity at 50uM, 25uM and 12.5uM and pro-cathelicidin at highest concentration tested of 50uM. hCLD shows no antibacterial activity in this assay.

1. Ericksen, B., Wu, Z., Lu, W., and Lehrer, R. I. (2005) Antibacterial activity and specificity of the six human {alpha}-defensins, *Antimicrob Agents Chemother 49*, 269-275.

2. Ganz, T., Selsted, M. E., Szklarek, D., Harwig, S. S., Daher, K., Bainton, D. F., and Lehrer, R. I. (1985) Defensins. Natural peptide antibiotics of human neutrophils, *The Journal of clinical investigation 76*, 1427-1435.

3. Lehrer, R. I., Rosenman, M., Harwig, S. S., Jackson, R., and Eisenhauer, P. (1991) Ultrasensitive assays for endogenous antimicrobial polypeptides, *J Immunol Methods 137*, 167-173.

4. Shi, J., Ross, C. R., Leto, T. L., Blecha, F. (1996) PR-39, a proline-rich antibacterial peptide that inhibits phagocyte NADPH oxidase activity by binding to Src homology 3 domains of p47 phox. *Proc. Natl. Acad. Sci.* 11;93(12):6014-8

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