Supplemental Information

Table S1. Data collection and refinement statistics

	Crystal	
	L-HNP1	D-HNP1
Data collection		
Space group	$P2_12_12$	$P2_12_12$
Cell parameters, Å	a=45.47, b=31.34, c=40.21	a=45.28 Å, b=31.10, c=40.28
Molecules/a.u.	2	2
Resolution, Å	50-1.56 (1.62-1.56)	50-1.56 (1.62-1.56)
Number of reflections		
Total	14,915	15,401
Unique	8,146	8,427
R_{merg}[b], %	7.0 (15.2)	7.8 (18.4)
Completeness, %	94.2 (85.9)	98.2 (92.5)
Redundancy	7.0 (6.9)	6.6 (6.4)
I/σ, I	16.0 (13.9)	17.4 (10.8)
Refinement Statistics		
Resolution, Å	30-1.56	12-1.56
R[c], %	17.1	18.9
R_{free}[d], %	19.5	19.9
Number of atoms		
Protein[e]	476	479
Water	61	47
Ligand	13	3
Root mean square deviation		
Bond lengths, Å	0.011	0.006
Bond angles, °	1.42	1.79

[a]All data (outer shell).
[b]$R_{merge} = \sum | I - \langle I \rangle | / \sum I$, where I is the observed intensity and $\langle I \rangle$ is the average intensity obtained from multiple observations of symmetry-related reflections after rejections
[c]$R = \sum \| F_o | - | F_c \| / \sum | F_o |$, where F_o and F_c are the observed and calculated structure factors, respectively
[d]R_{free} = defined by Brünger
[e]Numbers refer to non-H atoms. Differences result from different number of residues accommodating multiple conformations

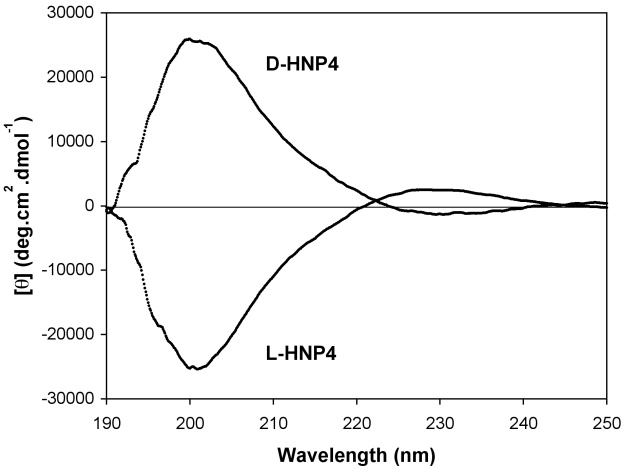

Figure S1. Circular dichroism spectra of ^{L}HNP4 and ^{D}HNP4 at 50 μM in 5 mM phosphate buffer, pH 7.4. The spectra were acquired at room temperature using a 1-mm cuvette.

Cytotoxicity of anthrax lethal toxin. One day before the assay, RAW 264.7 (ATCC TIB-71) cells were seeded in a 96-well plate at a density of 3 x 10^4 cells per well in RPMI medium 1640 supplemented with 5% FCS and appropriate amounts of antibiotics, and incubated in 5% CO_2 at 37 °C. 400 ng/ml lethal factor, 1600 ng/ml protective antigen and a two-fold dilution series of HNP1 (0, 1.25, 2.5, 5, 10, 20, 40, 80 μM), prepared in RPMI medium 1640 supplemented with 5% FCS, were added simultaneously to cells. Five hours after treatment, cell viability was determined using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay (Promega). Specifically, 100 μl of PMS solution was added to 2.0 ml of MTS solution. 20 μl of the combined MTS/PMS solution was added into each well of the 96 well assay plate containing 100 μl of cells in culture medium. The cells were further incubated for 1 hour, and the absorbance at 490 nm was recorded using an ELISA plate reader.

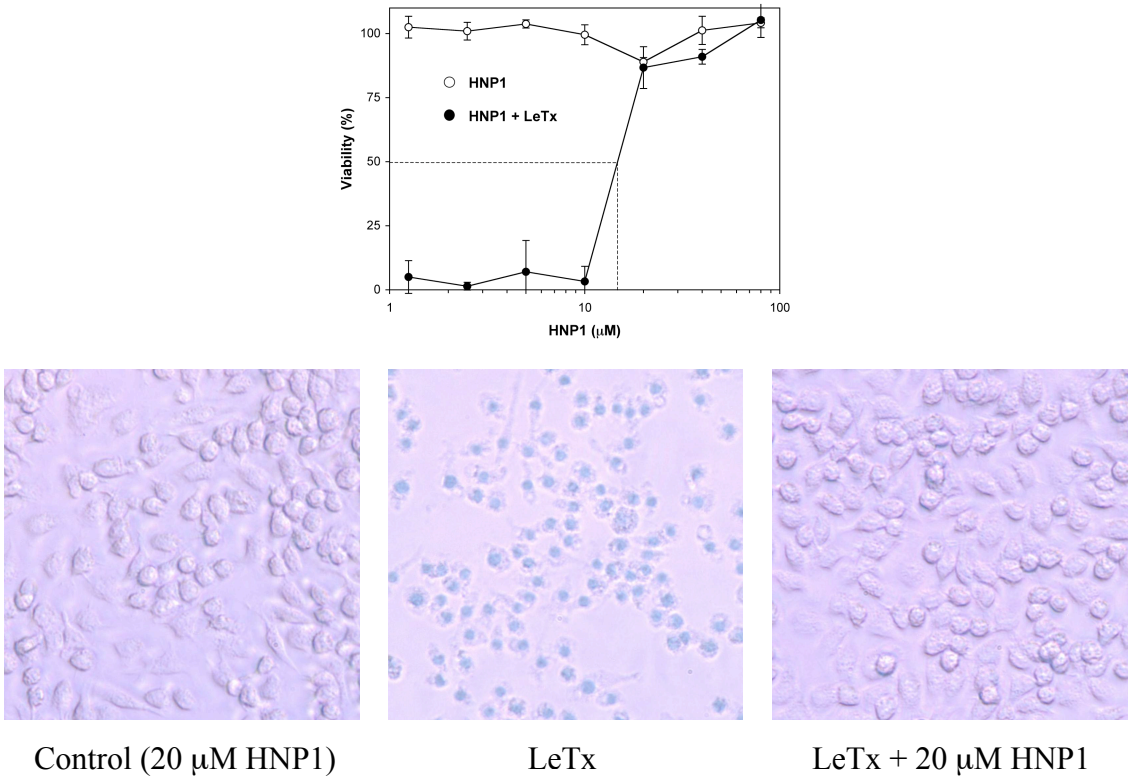

Figure S2. Neutralization of anthrax lethal toxin (LeTx) by synthetic HNP1. Top panel: dose-dependent protection of macrophages against cytolysis by LeTx in RPMI medium 1640 supplemented with 5% FCS. Bottom panel: trypan blue cell staining experiments showing that 20 μM HNP1 fully protected macrophages from anthrax LeTx-induced cytolysis.