Supporting Information

Python cathelicidin CATHPb1 Protects against Multidrug-Resistant Staphylococcal Infections by Antimicrobial-Immunomodulatory Duality

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Contents：

Supplementary materials and methods

Gene cloning and characterization of CATHPbs.

One young male *Python molurus bivittatus* was collected from Dalian, Liaoning Province, China. After diethyl ether anesthesia, the snake was killed and the tissue sample of lung was dissected immediately and frozen in liquid nitrogen for use. Total RNA of boa lung tissue was extracted using the AxyPrep^{TM} Multisource Total RNA Miniprep Kit (Invitrogen, USA). All of the animal experiments were approved by Animal Care and Use Committee of Dalian University of Technology. Considering the close evolutionary relationship between the king cobra and boa, the king cobra cathelicidin sequence (Accession: ACF21002) was used to search cathelicidin-like sequence in boa genome by BLAST at National Center for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The complete cathelicidin sequence of partial hits, found with genomic mining, was obtained by means of 3'RACE-PCR methods (GeneRacer^{TM} Kit, Invitrogen) according to the manufacturer's instructions. 1 μg total RNA from boa lung tissue was used to synthesize the 3' RACE ready first-strand cDNA by Cloned Avian Myeloblastosis Virus Reverse Transcriptase (Cloned AMV RT), the primer used was GeneRacer^{TM} Oligo dT Primer (5′-GCTGTCAACGATACGCTACGTAACGGCATGACAGTG$(T)_{18}$-3′). To obtain the 3' end of the partial hit, two forward gene-specific primers (GSP and NGSP, Table S5) were designed on basis of the known 5'-sequence and used to amplify the first-strand cDNA by Nest-PCR in combination with the GeneRacer^{TM} 3' Primer

(5′-GCTGTCAACGATACGCTACGTAACG-3′) and GeneRacer™ 3’ Nested Primer (5′-CGCTACGTAACGGCATGACAGTG-3′), respectively. The nested PCR contained two parts of outer PCR and inner PCR. GSP coupled with GeneRacer™ 3’ Primer were used in outer PCR. The procedure of outer PCR was 3min at 94℃； 25 cycles: 30 s at 94℃, 30 s at 56℃ and 30 s at 72℃；following by an extension step at 72 ℃ for 10 min. The inner PCR using the NGSP and GeneRacer™ 3’ Nested Primer was 3 min at 94℃； 30 cycles: 30 s at 94℃, 30 s at 58℃ and 30 s at 72℃； following by an extension step at 72℃ for 10 min. The inner PCR product was then purified and cloned into the pMD19-T Vector (Takara, Japan) for sequencing.

Peptides synthesis and purification

Peptides were synthesized by Fmoc chemistry-based solid-phase synthesis on an automated peptide synthesizer (GL Biochem Ltd., Shanghai, China). Briefly, C-terminal amino acid Carboxyl of the target peptide was covalent coupled to an insoluble polymer resin, and a stepwise elongation of peptide chains proceeded smoothly with N alpha-9-fluorenylmethoxycarbonyl (Fmoc) amino acid derivatives using a carbodiimide/HOBt mediated reaction. The final cleavage of side-chain protecting groups and the release of the C-terminal amide moiety was achieved by the treatment with trifluoroacetic acid, dichloromethane in the presence of scavengers.

The purity (>95%) and identity of each synthetic peptide was then confirmed by high-performance liquid chromatography (HPLC) and electronic spray ionization-mass spectrometry (ESI-MS). Briefly, peptides were analyzed by Agilent 1100 HPLC system

performed on a YMC-Triart C18 column (4.6×250 mm), pre-equilibrated with 0.1% trifluoroacetic acid in water. Elution was achieved at a flow rate of 1.0 mL/min by a linear gradient of acetonitrile in 0.1% trifluoroacetic acid in water (0-30 min, 10-50% acetonitrile). Mass spectrometry analysis was performed on a LCQ Fleet Ion Trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an electrospray ion source in positive ion mode. All procedures were carried out according to manufacturer's standard protocols, and the data were analyzed by the software package provided by the manufacturer. All peptides synthesized were dissolved in distilled water for assays.

Antimicrobial and bacterial killing kinetics assays

Antimicrobial activity of CATHPbs was determined according to our previously described methods [1-4]. More than 40 strains including standard and clinically isolated drug-resistant strains of bacteria and fungi were evaluated in this study. Briefly, all the strains were cultured in MH broth at 37°C to logarithmic phase, and diluted to 2×10^5 cells/mL for use. A serial dilution of CATHPbs were prepared in 96-well microtiter plates and mixed with equal volume of bacterium suspension. The plates were then put into microbial incubator and slowly shaken at 70 rpm for 16-18 h. At the time points, the plates were measured at OD600 nm by a Varioskan® Flash fluorescence microplate (Thermo Scientific, USA). The minimal concentrations at which no microbial growth occurred were recorded as MIC (minimal inhibitory concentration) values. Three traditional antibiotics, cefoperazone, vancomycin and oxacillin were used as positive

control. The MIC values were expressed as μg/mL in three independent experiments.

For bacterial killing kinetics assay, *S. aureus* ATCC25923 was cultured over-night and diluted to 1×10^5 cells/mL in MH broth. A final 5×MIC concentration of CATHPb1 was added into the bacterial suspension and incubated for 0, 10, 20, 30, 45, 60, 90, 120 and 180 minutes at 37℃. At indicated time points, 10 μL aliquot was extracted and diluted with MH broth for 100 times. 100 μL of the diluent was then coated on MH agar plates to assess the surviving bacteria. The antibiotic vancomycin (5×MIC concentration) and sterile deionized water were used as positive and negative control, respectively.

Measurement of intracellular reactive oxygen species

Intracellular ROS generation after exposure to various concentration of CATHPb1 (10, 20 and 40 μg/mL) was measured using 2,7-dichlorofluorescin diacetate (DCFH-DA; Sigma, USA). Briefly, cells were diluted to 1×10^6 cells/mL by RPMI 1640 and seeded in 96-well black culture plates for 100 μL /well. After adherence, the cells were stimulated by CATHPb1 for 24 h, washed three times by PBS and incubated with DCFH-DA（20 μmol）for 30 min at 37℃. Fluorescence was measured on a Varioskan® Flash fluorescence microplate (Thermo Scientific, USA) with excitation wavelength 488 nm and emission wavelength 525 nm. The values were expressed as a percent of fluorescence intensity relative to the control wells.

Phagocytosis assay

The VRSA strains were diluted to 0.5×10^9 CFU/mL with carbonate buffer solution and loaded with 0.3 mg/mL FITC fluorescent dye (Sigma, USA) for 2 h at 37℃. After

washing twice with PBS, the bacteria were then fixed with 1% paraformaldehyde for 30 min and washed five times with fresh PBS. MPMs were pre-treated with 20 μg/mL CATHPb1 for 4 h, and then incubated with the FITC-labelled VRSA at a multiplicity of 30 for 2.5 h. Cells that were not incubated with FITC-conjugated VRSA strain were used as background. At the indicated time points, the extracellular fluorescence were quenched with 1 mg/mL trypan blue, and the cells were thoroughly washed with ice-cold PBS and analyzed by flow cytometry (FACSVantage SE, BD Biosciences, San Jose, CA).

Calcein leakage assay

The leakage of the preloaded fluorescent dye calcein-AM assay was performed as previously described with some modification [5]. *S. aureus* ATCC25923 was grown to logarithmic phase at 37℃ in MH broth. The cells were then collected and re-suspended in PBS (containing 10% MH broth) to an OD600 of 1.0 (~ 10^9 CFU/mL). The bacteria were then incubated with calcein-AM (Thermo Fisher, USA) with a final concentration of 3 μmol for 1 h at 37℃. After centrifugation at 5000 rpm for 10 min, the calcein-AM-loaded *S. aureus* were harvested and diluted to a final concentration of 10^7 CFU/mL for future use. CATHPb1 were added at a concentration equal to 5×MIC. Cells treated with Nisin (5×MIC concentration, Sigma, USA) and ddH_2O were positive and negative controls, respectively. The fluorescence intensity was monitored every 5 min within a 30 minute time span in a fluorescence plate reader (Varioskan® Flash, Thermo Scientific, USA), with excitation and emission wavelengths of 490 nm and 515 nm, respectively. The calcein leakage rate (%) was calculated as the absolute percent

peptide-induced calcein leakage relative to untreated calcein-loaded cells. Experiments were performed in triplicate.

Scanning electron microscopy (SEM)

SEM was performed as described previously [6]. Briefly, VRSA strains were cultured in MH broth to logarithmic phase, washed and resuspended in saline solution. The bacteria were incubated with CATHPb1 (5×MIC) at 37℃ for 1 h. After centrifuged at 8000 rpm for 4 min, the bacteria pellets were collected and fixed with 2.5% glutaraldehyde solution at 4℃ overnight. The bacteria were then dehydrated in a graded series of ethanol, frozen in liquid nitrogen-cooled terbutyl alcohol, and vacuum-dried overnight. Next, the sample powder was mounted onto aluminum stubs and vacuum sputter-coated with gold. The samples were then imaged using Nova NanoSEM™ 450 scanning electron microscope (FEI, USA) under standard operating conditions.

Cytotoxic and hemolytic assays

In vitro cytotoxicities of CATHPbs against human liver cells HL7702 and MPMs were determined by MTT described previously [7, 8]. Hemolytic assay was tested using fresh-prepared human red blood cells as reported previously [6]. The amount of hemoglobin released from lytic human erythrocytes was measured by monitoring the absorbance at 540 nm, using PBS and 1% Triton X-100 (v/v) as the blank and positive control, respectively. All experiments were performed in triplicate. The percentage of hemolysis was calculated as following: Percent hemolysis = $[(A_{sample} - A_{blank})/(A_{positive} - A_{blank})] \times 100\%$.

Structure analysis

The secondary structure of CATHPb1 was evaluated by Circular dichroism (CD) spectroscopy in a Jasco J-810 spectrophotometer (Jasco, Japan). Briefly, CATHPb1 (0.5 mg/mL) was prepared in TFE/H2O solutions (0~90%) or SDS (0~120 mM) to a concentration of 0.2 mg/mL, and then added into a quartz optical cell with a path length of 0.5 mm at 25°C. The CD spectra from 190 to 260 nm was measured at 298 K with the instrument parameters of 0.1 cm path-length cell, 0.2 nm resolution and a scan speed of 100 nm/min. The spectra were averaged over three consecutive scans, followed by subtraction of the CD signal of the solvent. The amphipathicity of peptide was analyzed by plotting the helical wheel diagrams online (http://heliquest.ipmc.cnrs.fr/cgi-bin/ComputParamsV2.py). The 3D structure of CATHPb1 was manually built using Rosetta *ab initio* software (version 3.5). The lowest energy structure from 5000 decoys was verified by PROCHECK. The generated 3D structure of CATHPb1 was then visualized by PyMol software without any other refinement.

Reference:

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Supplementary Tables

Table S1. Physicochemical and structural properties of CATHPbs.

Peptides	Sequences	Net charge	Theoretical PI	Mw	Helix[a] (%)
CATHPb1	KRFKKFFRKIKKGFRKIFKKTKIFIGGTIPI(31aa)	+13	12.33	3800.82	74.2%
CATHPb2	KRNGFRKFMRRLKKFFAGGGSSIAHIKLH(29aa)	+9	12.49	3389.08	51.7%
CATHPb3	KRFQNFFRELEKKFREFFRVYRITIGATIRF(31aa)	+6	11.38	4044.77	77.4%
CATHPb4	TRSRWRRFIRGAGRFARRYGWRIALGLVG(29aa)	+9	12.54	3492.11	86.2%
CATHPb5	SPPQAMGFPPQVNVEHYIPASYSVAALTVTEEE(33aa)	-4	4.09	3559.95	0%
CATHPb6	RAAPQRRLRAMARLKKFAEAGGADPDSGGLRARFPER(37aa)	+6	11.79	4080.69	45.9%

[a]: The α-helix content of CATHPbs were predicted by PSIPRED v3.3 server (http://bioinf.cs.ucl.ac.uk/psipred/).

Table S2. *In vitro* antimicrobial activity of CATHPbs.

Microorganism Strains	MIC[a] (μg/mL)					
	CATHPb1	**CATHPb2**	**CATHPb4**	**CPZ/SBT**	**Vanc**	**Oxa**
Gram-positive bacteria						
Staphylococcus aureus (ATCC25923, MSSA)	37.5	-[b]	18.75	0.29	0.29	2.34
S. aureus (IS[c] 08032712, MRSA)	37.5	-	18.75	-	0.59	-
S. aureus (IS 08032810, VRSA)	4.69	-	18.75	18.75	-	-
S. aureus (IS 08032706)	18.75	75	18.75	18.75	0.59	2.34
Staphylococcus epidermidis	18.75	75	18.75	-	-	-
Nocardia asteroids	9.38	37.5	9.38	18.75	0.29	18.75
Bacillus cereus	1.17	9.38	9.38	-	-	-
Enterococcus faecalis (IS 981)	75	-	37.5	18.75	0.29	18.75
Enterococcus faecium (IS 1299)	9.38	18.75	18.75	18.75	-	-
Gram-negative bacteria						
Escherichia coli (ATCC25922)	9.38	37.5	18.75	0.29	-	4.69
E. coli (IS 08040726)	9.38	75	18.75	4.69	-	-
E. coli (IS 08032813)	9.38	75	18.75	4.69	-	-
Klebsiella oxytoca	75	-	18.75	-	-	-
Salmonella paratyphi (IS 738)	18.75	75	37.5	-	-	-
Dysentery bacillus	1.17	2.34	4.69	18.75	-	4.69
Klebsiella pneumoniae (IS 08B343)	18.75	75	18.75	37.5	-	-
K. pneumoniae (IS 1400)	18.75	-	37.5	37.5	-	-
Stenotrophomonas maltophilia (IS 090223)	4.69	9.38	18.75	18.75	-	-
Pseudomonas aeruginosa ATCC 27853	9.38	-	18.75	0.59	-	4.69
P. aeruginosa (IS 08031014)	37.5	-	9.38	18.75	-	-
Fungi						
Candida albicans (IS 08022710)	18.75	37.5	18.75	18.75	-	-
C. albicans (IS 08030401)	9.38	37.5	9.38	18.75	-	-

C. albicans (IS 08022821)	18.75	37.5	-	18.75	-	-
C. albicans (IS 08030809)	18.75	37.5	18.75	4.69	-	-
C. albicans (IS 08030102)	18.75	18.75	18.75	9.38	-	-
Candida glabrata (IS 08A802)	9.38	37.5	9.38	1.17	-	-
C. glabrata (IS 09050201)	18.75	75	18.75	0.59	-	75

[a] MIC: minimal inhibitory concentration;. These concentrations represent mean values of three independent experiments performed in duplicates. [b] -: no detectable activity in MIC assay in dose of 100 μg/mL; [c] IS: clinically isolated drug-resistance strain. CPZ/SBT: cefoperazone/sulbactam, 1:1; Vanc: vancomycin; Oxa: oxacillin; MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus*; VRSA: vancomycin-resistant *S. aureus*. CATHPb 3, 5 and 6 didn't show any detectable activities against all the tested microorganisms even at the concentration up to 100 μg/mL (data not shown).

Table S3. Toxicity of CATHPbs and LL-37.

Cells	Toxicity (%)[a]						
	CATHPb1	**CATHPb2**	**CATHPb3**	**CATHPb4**	**CATHPb5**	**CATHPb6**	**LL-37**
Human Erythrocytes[b]	6.63	7.28	9.14	65.59	6.89	7.14	6.49
HL7702[c]	4.46	7.5	8.42	8.74	7.59	9.62	7.84
Mouse Peritoneal Macrophages[c]	6.46	6.7	4.65	12.95	9.86	6.47	7.32
LAD2[d]	10.97	25.28	11.37	22.45	10.73	16.29	35.85

[a]: The concentrations of CATHPbs and LL-37 used in this assay were 100 μg/mL; [b]: Hemolysis of CATHPbs and LL-37 against human erythrocytes; [c]: Cytotoxicity of CATHPbs and LL-37; [d]: Mast cell degranulation in response to CATHPbs and LL-37 determined by the release of β-hexosaminidase; LL-37, the human cathelicidin, was used as control. Results represent the mean values of five independent experiments.

Table S4. The anti-biofilm activity of CATHPb1.

Bacterial strains	**BIC-2[a] (μg/mL)**	**BIC-5[b](μg/mL)**	**BEC-2[c] (μg/mL)**
E. coli ATCC 25922	2.5	3.1	37.5
K. oxytoca	6.25	10	25
P. aeruginosa ATCC 27853	6.25	10	44
S. aureus IS 08032706	7	18.7	30.5
S. aureus IS 08032810	11.8	31	55.3
C. albicans IS 08030809	4.2	8.7	71
C. glabrata	3.2	8.7	60

[a] BIC-2 and [b]BIC-5: minimal concentrations resulting in 50% and 80% decrease in biofilm formation compared with the control. [c] BEC-2: minimal concentrations leading to 50% eradication of mature biofilm.

Table S5. Primers used in qPCR and RACE-PCR assays.

Name	Forward (5'-3') in qPCR	Reverse (5'-3') in qPCR
TNF-α	CGGTGCCTATGTCTCAGCCT	GAGGGTCTGGGCCATAGAAC
IL-1β	ATGGCAACTGTTCCTGAACTC	GCCCATACTTTAGGAAGACA
IL-6	AGTTGCCTTCTTGGGACTGA	TCCACGATTTCCCAGAGAAC
iNOS	CTGCAGCACTTGGATCAGGAACCTG	GGAGTAGCCTGTGTGCACCTGGAA
IL-10	GGTTGCCAAGCCTTATCGGA	GAGAAATCGATGACAGCGCC
MCP-1/CCL2	GCTGTAGTTTTTGTCACCAAGC	GTGCTGAAGACCTTAGGGCA
MIP-2/CXCL2	CCAGACAGAAGTCATAGCCACT	GGTTCTTCCGTTGAGGGACA
IFN-β	GGAAAGATTGACGTGGGAGA	CTGAGGCATCAACTGACAGG
GAPDH	GTGAAGGTCGGTGTGAACGGATT	GGAGATGATGACCCTTTTGGCTC
	GSP (5'-3') in RACE-PCR	**NGSP (5'-3') in RACE-PCR**
XM_007445200.1	CTGTGGCTCTTGGCATGGAACT	TGTGCCTGGTCCAAGAAGAACG
XM_007444974.1	GCTGAAGTTCACCATCAAGGA	TACTCTACGCAACAAGAGC

Supplementary Figures

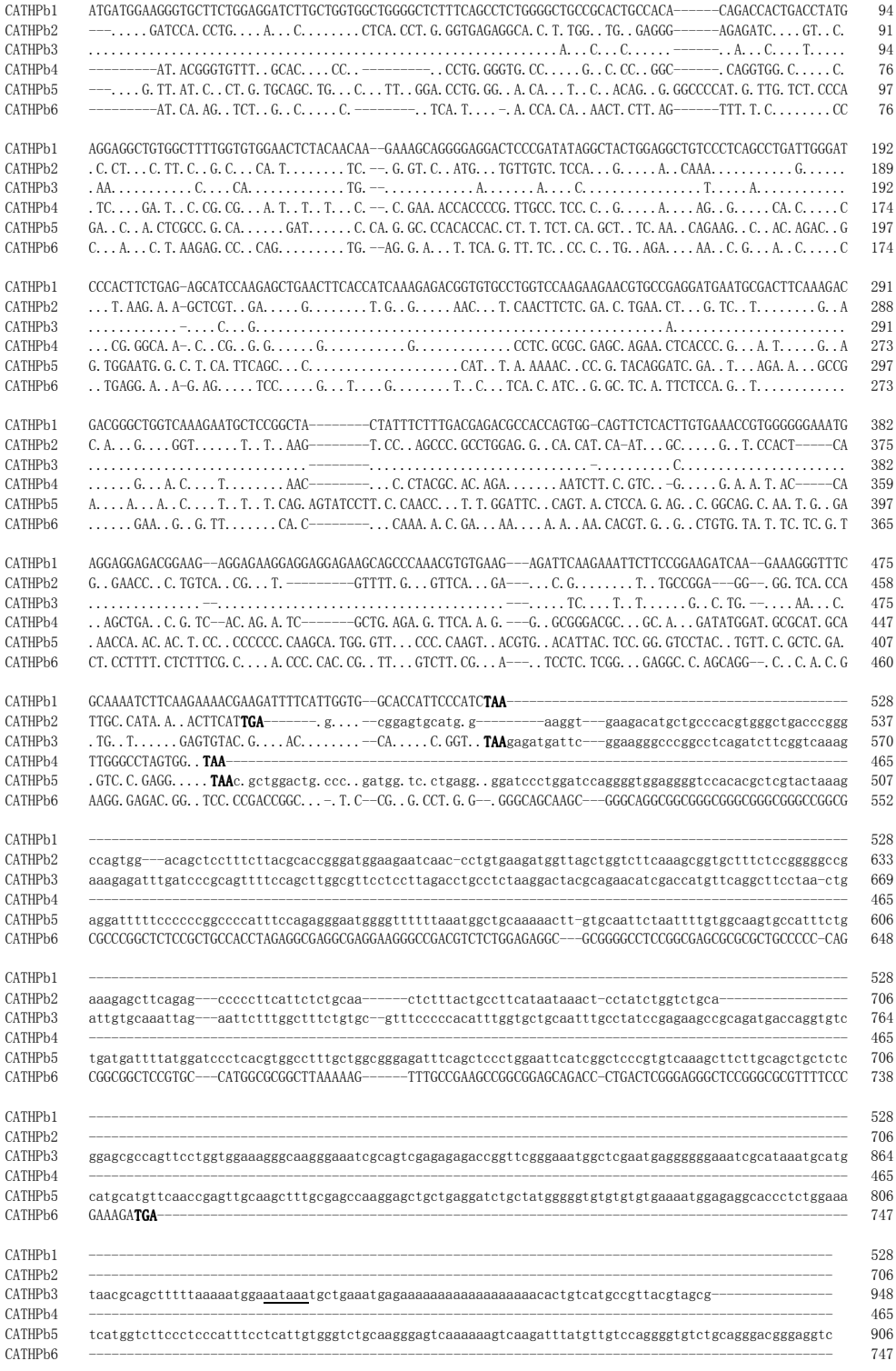


Figure S1. Alignment of cDNA sequences encoding CATHPbs precursors. The stop codons TGA and TAA are in bold. The potential polyadentlation signal "aataaa" is underlined. The 3'- UTR is shown in lower case. Dots represent similar base sequence. Dashes are inserted to maximize the similarity.

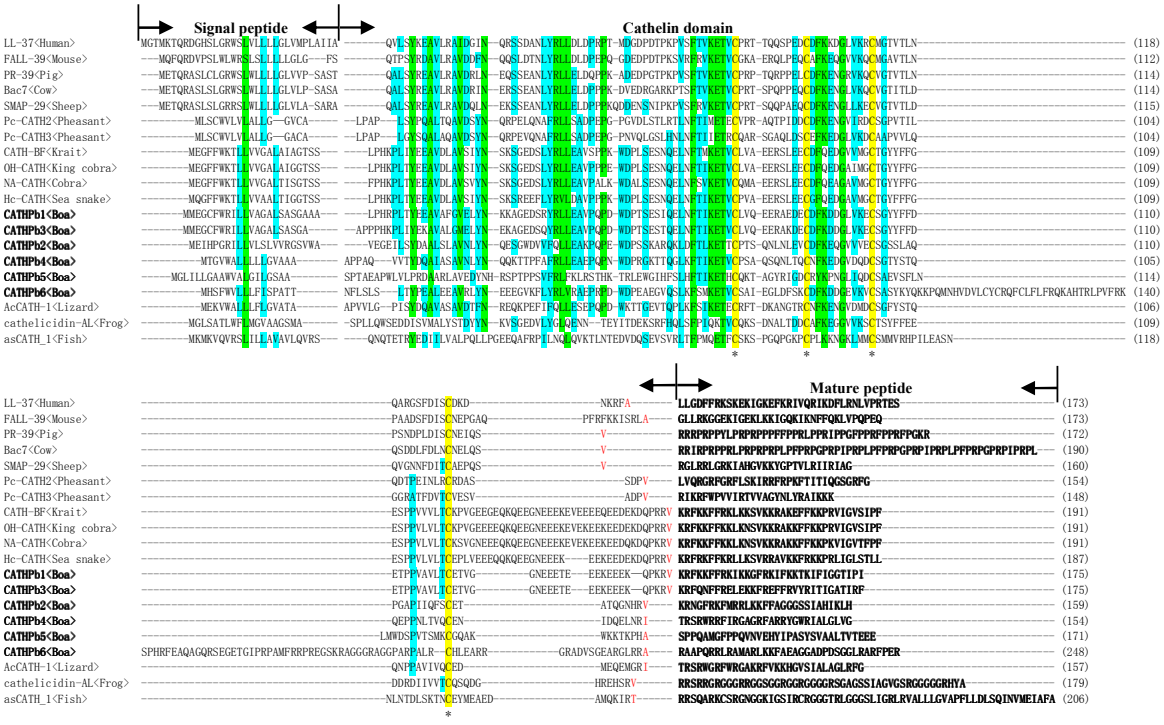
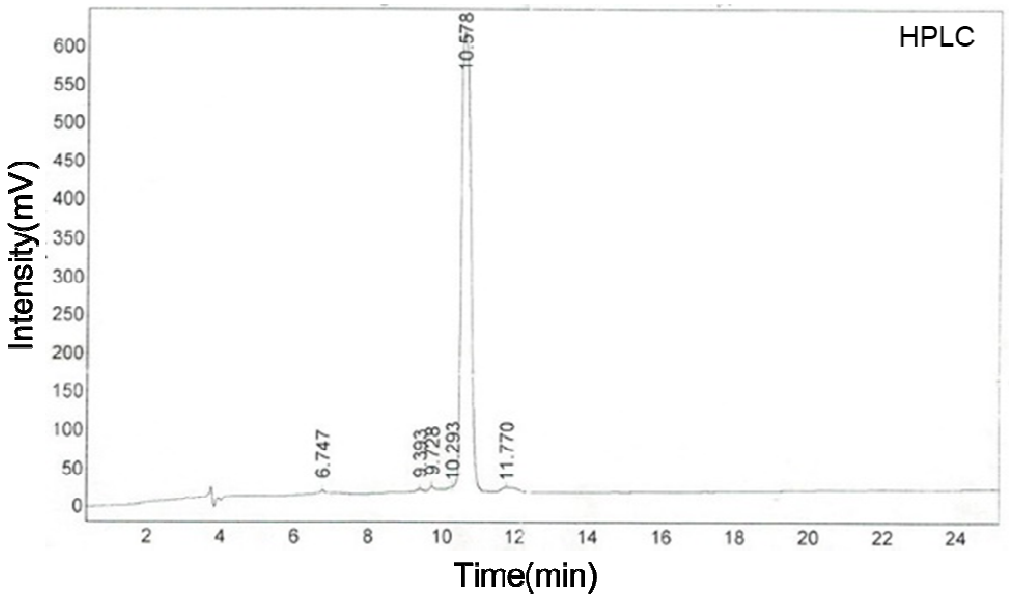


Figure S2. Multi-sequence alignment of CATHPbs precursor with other representative cathelicidins. The conserved residues are shaded. The four conserved cysteine residues in cathelin domain are indicated in asterisk (*). Elastase-sensitive residues of cathelicidins are marked in red. Mature peptides are indicated in bold. Dashes are inserted to maximize the similarity.



Peak No.	Time	Height	Area	Conc.
1	6.747	4323.296	35694.391	0.3204
2	9.393	3366.638	57877.828	0.5195
3	9.728	6602.026	112665.266	1.0113
4	10.293	6969.929	71617.375	0.6428
5	10.578	599105.500	10671976.000	95.7916
6	11.770	6235.952	190996.422	1.7144
Total		626603.341	11140827.281	100.0000

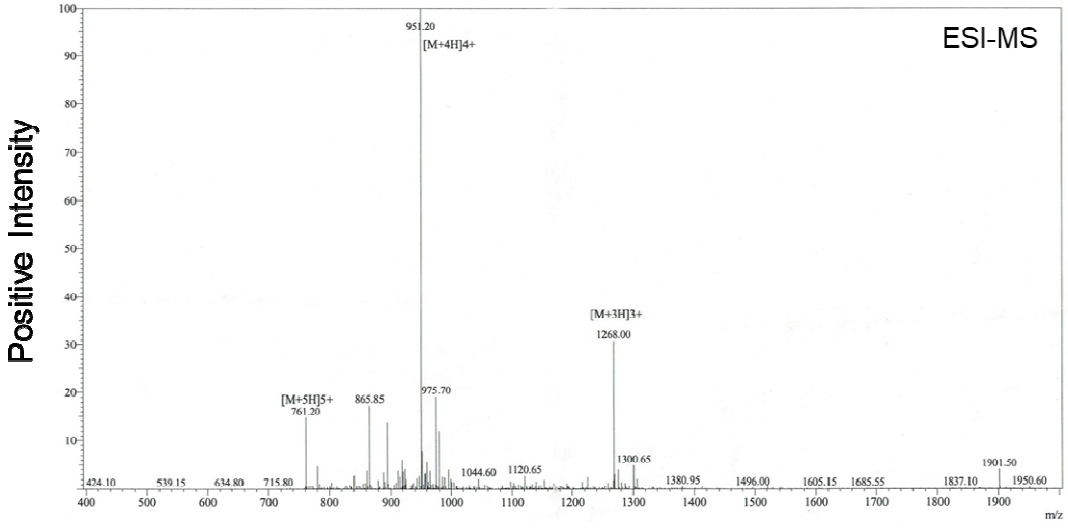
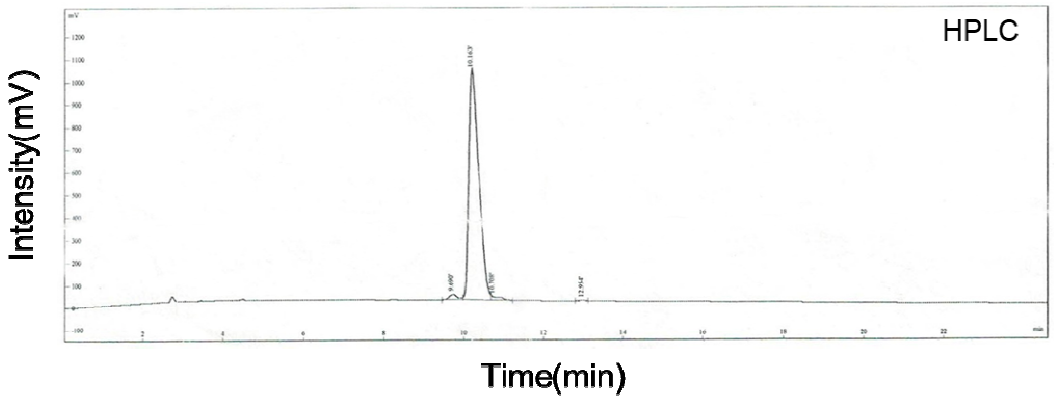


Figure S3A. The purity and identity of CATHPb1 was confirmed by HPLC and ESI-MS.



Peak No.	Time	Height	Area	Conc.
1	9.690	24734	350017	1.869
2	10.163	1026649	18084221	96.58
3	10.708	14697	267366	1.428
4	12.954	2669	24418	0.1304
Total		1068749	18726022	100.000

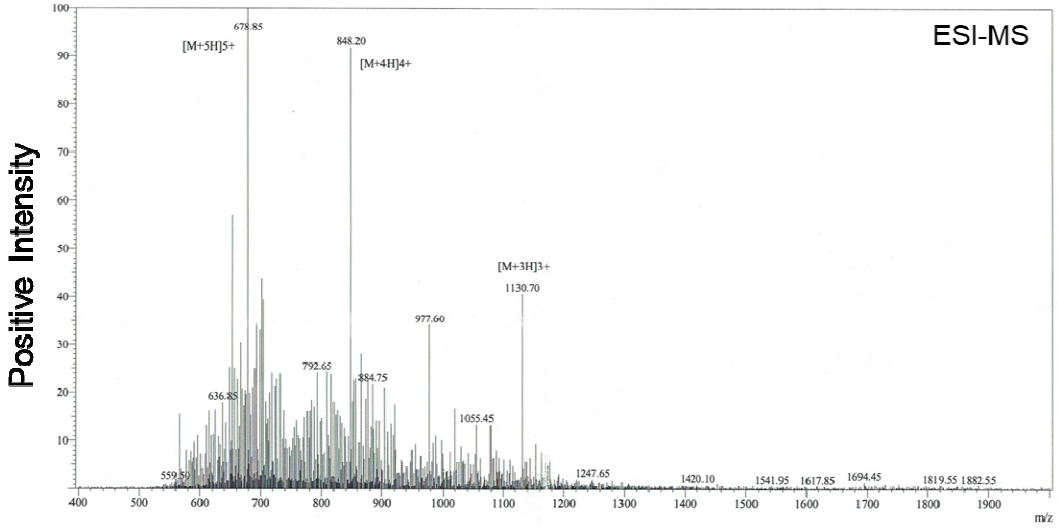
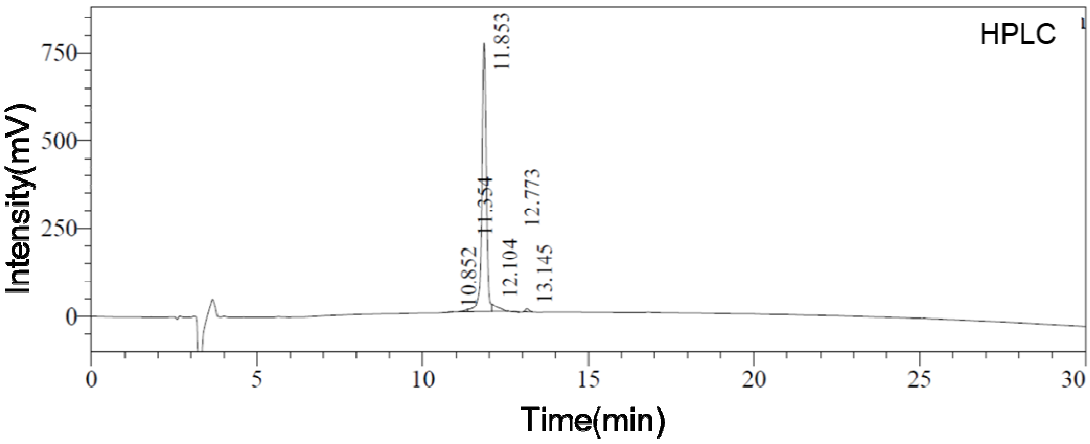


Figure S3B. The purity and identity of CATHPb2 was confirmed by HPLC and ESI-MS.



Peak No.	Time	Height	Area	Conc.
1	10.852	795	4728	0.062
2	11.354	6375	43004	0.562
3	11.853	764705	7264574	95.020
4	12.104	21963	261477	3.420
5	12.773	828	5478	0.072
6	13.145	8659	66036	0.864
Total		803326	7645297	100.000

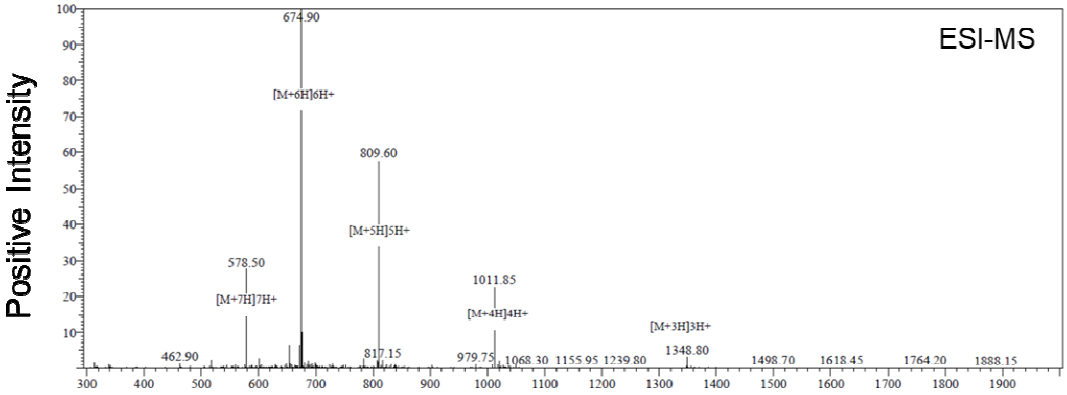
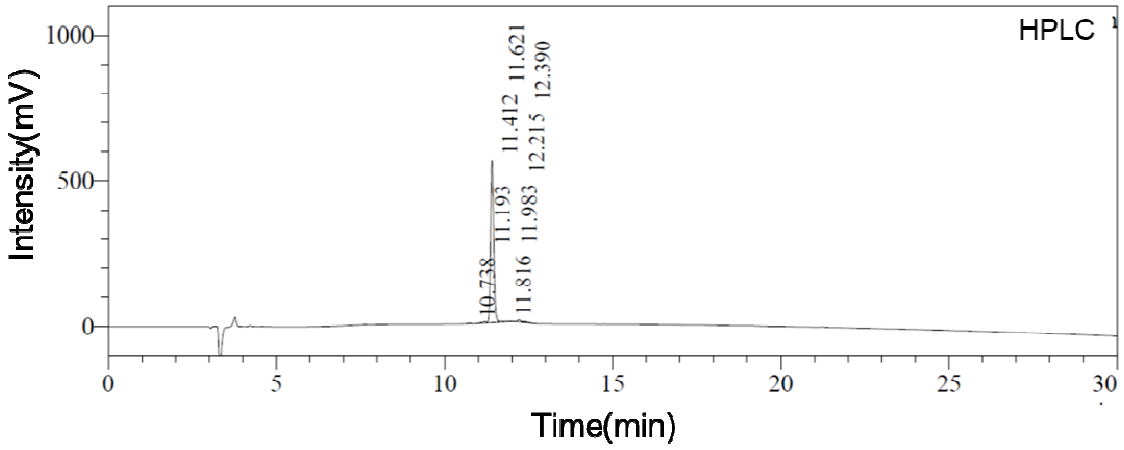


Figure S3C. The purity and identity of CATHPb3 was confirmed by HPLC and ESI-MS.



Peak No.	Time	Height	Area	Conc.
1	10.738	2370	12398	0.320
2	11.193	4180	39920	1.030
3	11.412	558134	3681767	95.022
4	11.621	3888	26703	0.689
5	11.816	3095	20271	0.523
6	11.983	1915	12715	0.328
7	12.215	7192	47688	1.231
8	12.390	3785	33171	0.856
Total		584558	3874631	100.000

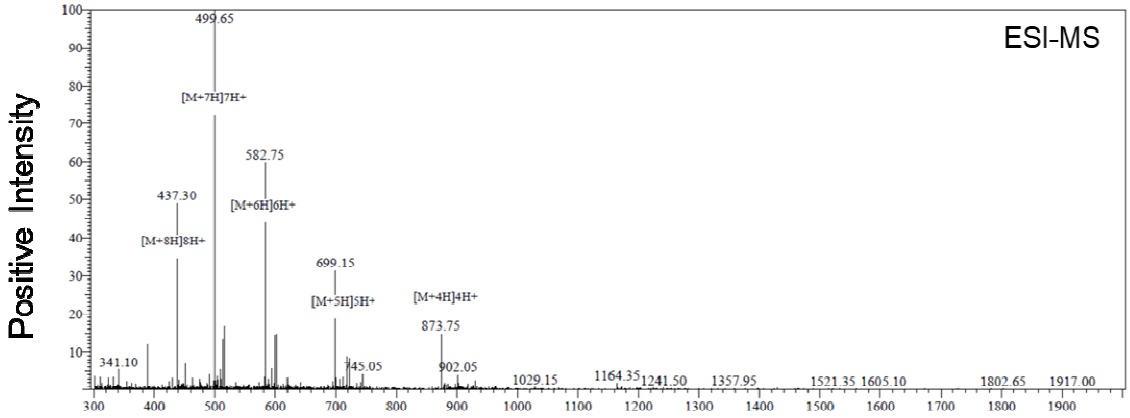


Figure S3D. The purity and identity of CATHPb4 was confirmed by HPLC and ESI-MS.

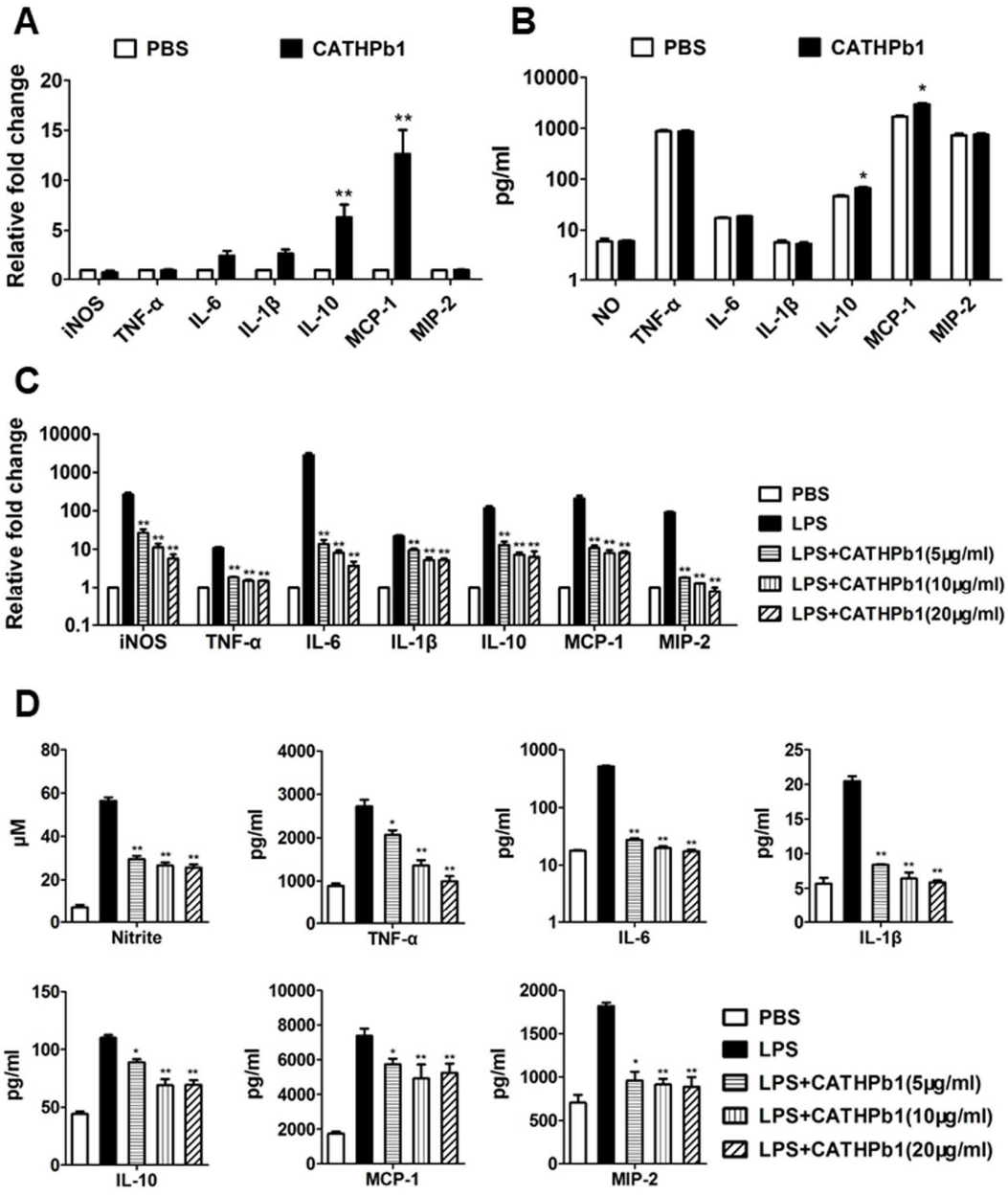


Figure S4. CATHPb1 selectively modulates NO and cytokines/chemokines production in MPMs. (A) CATHPb1 (20 μg/mL)-induced iNOS and cytokine/chemokine expression in MPMs was determined by qPCR. Expression of the target genes in the samples was normalized against the expression of GAPDH, and gene expression in untreated cells was normalized to 1. (B) CATHPb1-induced NO and cytokine/chemokines in MPMs culture supernatant were determined by Griess and ELISA. MPMs were stimulated with CATHPb1 or PBS for 24 h. (C-D) Effects of CATHPb1 on the TLR-mediated immune response to LPS (100 ng/mL), determined by qPCR (C) and ELISA (D). All data represents the mean ± SEM value of three independent experiments. * $P<0.05$, **

P<0.01, by unpaired t test.

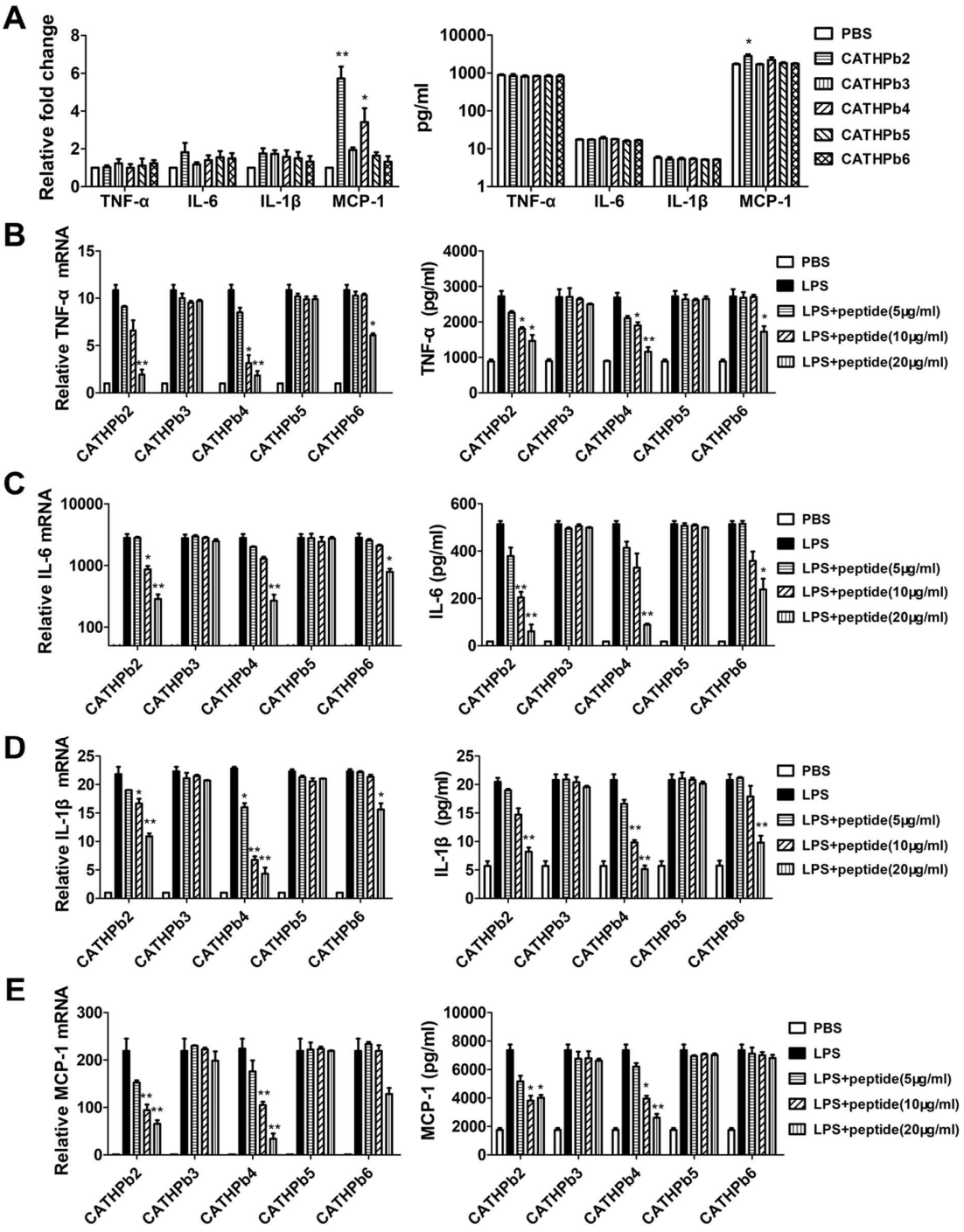


Figure S5. CATHPb2-6 selectively modulates cytokines/chemokine expression in MPMs. (A) CATHPb2-6 (20 μg/mL)-induced cytokine /chemokine transcription and production in MPMs. (B-E) Effects of CATHPb2-6 on cytokines/chemokine transcription and production of MPMs in response to LPS. (B) TNF-α; (C) IL-6; (D) IL-1β; (E) MCP-1.

Expression of the target genes were determined by qPCR, normalized to GAPDH and presented as the fold change relative to the gene expression in PBS blank group (normalized to 1) using the comparative △△Ct method. The production of cytokines and chemokine in cell culture supernatant were determined by ELISA. Data represents the mean ± SEM value of three independent experiments. * P<0.05, ** P<0.01, by unpaired t test.

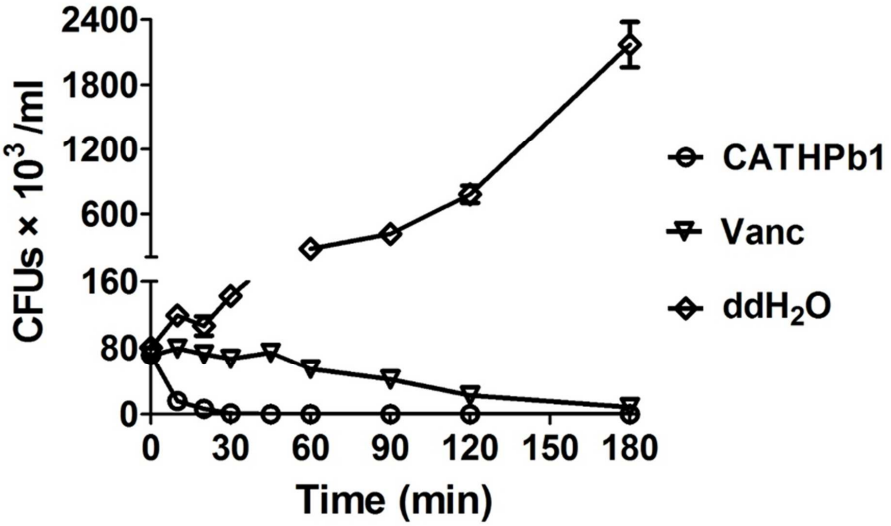


Figure S6. Time-killing kinetics of CATHPb1. 5×MIC of CATHPb1 and Vanc were used in this assay. Results represent mean values of three independent experiments.

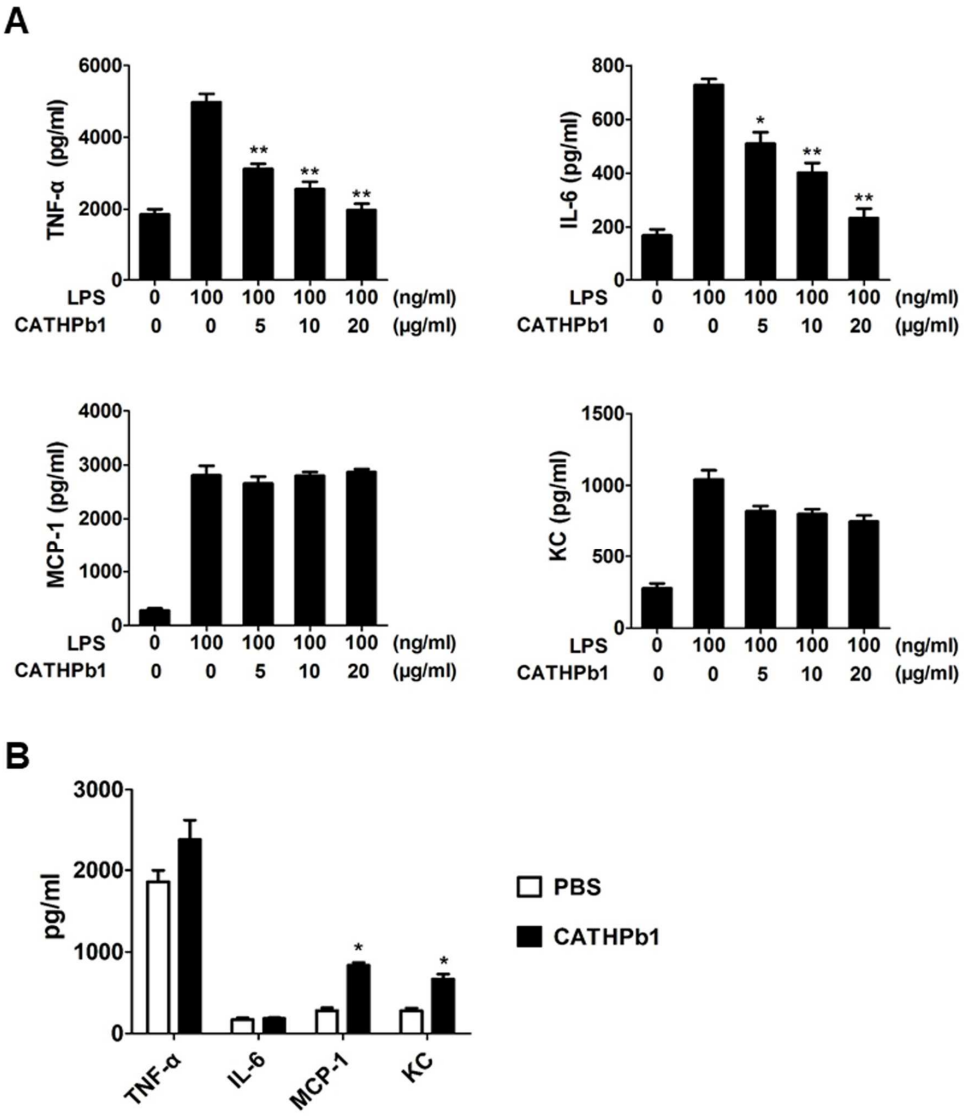


Figure S7. CATHPb1 selectively modulates inflammatory cytokines/chemokines production in neutrophils. (A) CATHPb1 of various concentrations modulated cytokine/chemokine productions stimulated by LPS (100 ng/mL) in neutrophils, determined by ELISA. (B) Neutrophils were treated with 20 μg/mL CATHPb1 for 12 h at 37℃. The cytokine/chemokine productions in cell culture supernatants were measured by ELISA. Data represents the mean ± SEM value of three independent experiments. * $P<0.05$, ** $P<0.01$, by unpaired t test.

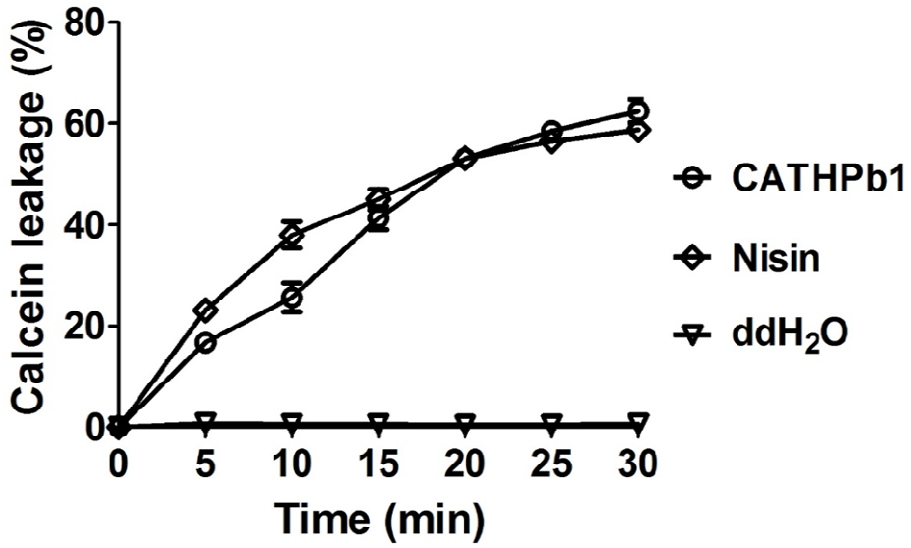


Figure S8. Effect of CATHPb1 on cytoplasmic membrane of *S. aureus* ATCC25923. The bacterial membrane permeabilization was indicated by the percentage of calcein-AM leakage after 30 min bacteria incubation with 5×MIC of CATHPb1, with Nisin and ddH_2O as positive and negative control, respectively. Data represents the mean ± SEM value of three independent experiments.

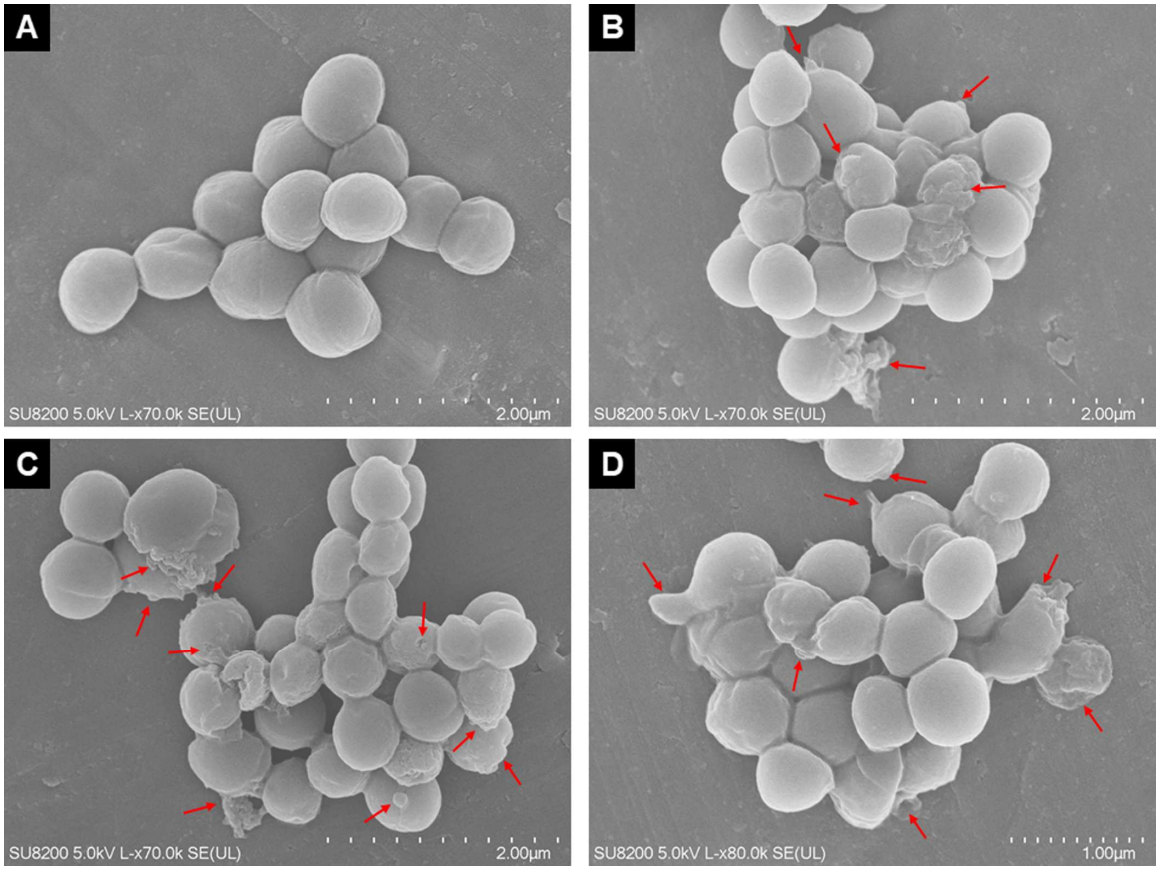


Figure S9. Effects of CATHPb1 on membrane morphology of VRSA strain analyzed by scanning electron microscopy (SEM). (A) PBS-treated bacteria. (B-D) CATHPb1-treated bacteria. The damage of microbial membranes and the efflux of the cellular inclusions are indicated by arrows.

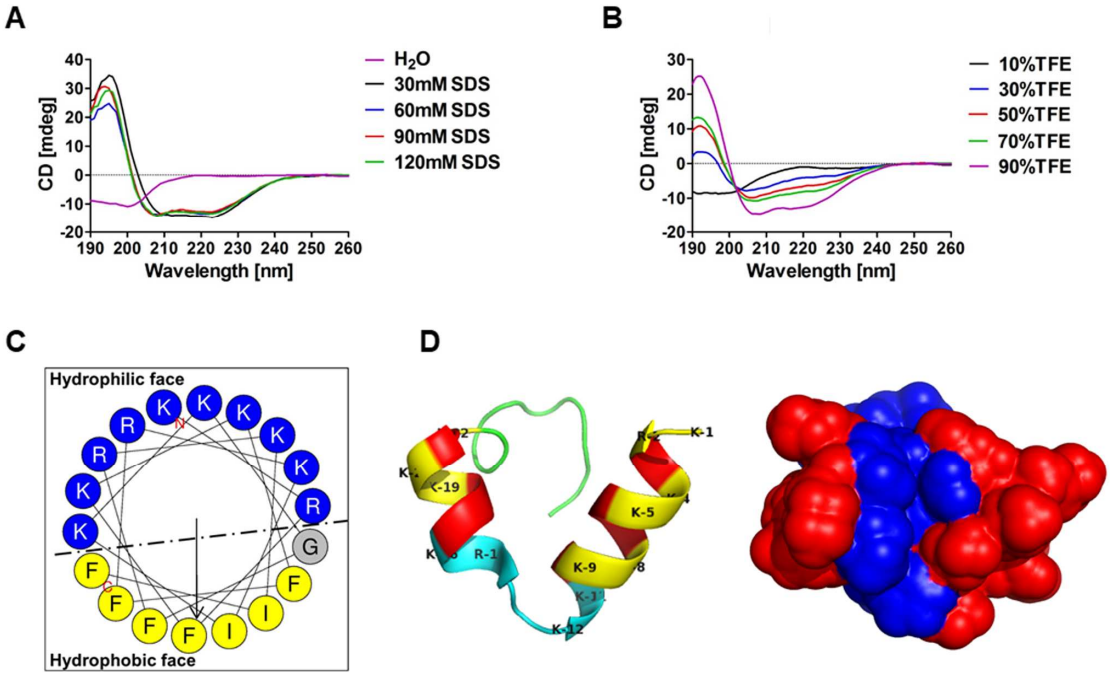


Figure S10. Structure characterizations of CATHPb1. Circular dichroism spectra of CATHPb1 in different membrane-like solvent environments: 0 ~120 mM SDS micelles (A) or 0~90% TFE (B). (C) Helix-wheel plots of CATHPb1. The hydrophobic and hydrophilic faces were separated by dash dot line, with the hydrophilic residues being concentrated on upper side of the helix and hydrophobic ones on the lower. (D) Tertiary structures built by Rosetta *ab initio* of CATHPb1. The KKGFRK sequence was labelled in cyan. Surface representation of CATHPb1 was shown in blue with the positive charged residues in red.

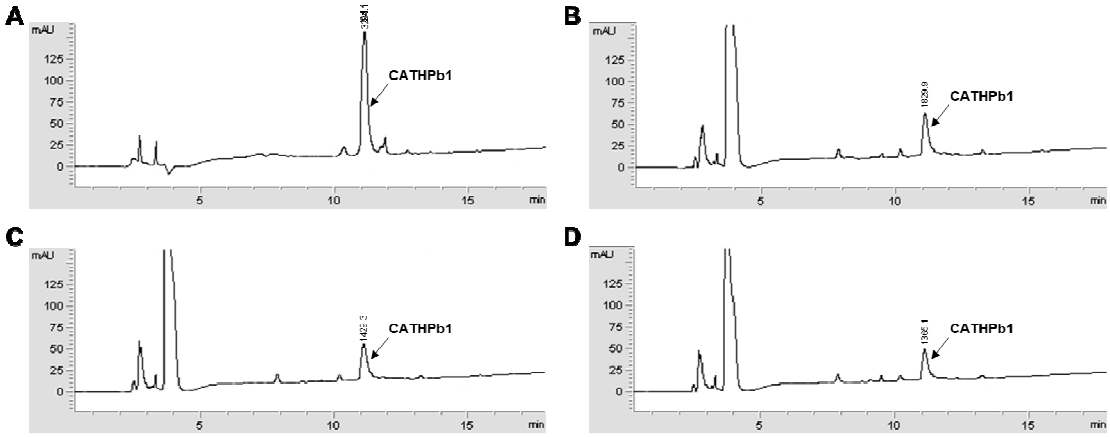


Figure S11. Analytical reversed-phase HPLC traces of CATHPb1 after treatment with 80% serum for different times. (A) HPLC analysis of pure CATHPb1. HPLC analysis of CATHPb1 after incubation with serum at 37□ for 1 h (B), 4 h (C) and 8 h (D).