Supporting Information

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SI Materials and Methods

Antibodies, ELISA Kits, PCR primers, Inhibitors, and siRNAs. Rabbit polyclonal antibody against βγ-crystallin fused aerolysin-like protein and trefoil factor complex (βγ-CAT) was produced as previously described (1). Rabbit polyclonal antibody against *Bombina maxima* IL-1β and Caspase-1 p20 subunit were produced as previously described (2). Rabbit polyclonal antibody against caspase-1 p10 (sc-515), IL-1β (sc-7884), NLRP3 (sc-134306), NLRC4 (sc-99055) ASC (sc-172054), and β-actin (sc-1615) were purchased from Santa Cruz Biotechnology. Rabbit polyclonal antibody against lamp-1 (ab24170) was purchased from Abcam. Mouse monoclonal antibody against lamp-1 (ab25630) was purchased from Abcam. Rabbit polyclonal antibody against Cathepsin B (12216-1-AP) was purchased from Proteintech Group. FITC-conjugated anti-mouse Gr-1 antibody (108405) and PE-conjugated anti-mouseF4/80 antibody (123109) were purchased from Biolegend.

ELISA kits for detecting mouse IL-1β, CXCL1, and CXCL2 were purchased from R&D Systems. A CytoTox 96 Non-Radioactive Cytotoxicity Assay kit for LDH assaying was purchased from Promega.

Caspase-1 inhibitor Ac-YVAD-cmk (sc-300323) was purchased from Santa Cruz Biotechnology. Nocodazole, bafilomycin A1, cytochalasin D, and CA074-me were purchased from Sigma-Aldrich.

siRNA targeting NLRP3 (sc-45470), NLRC4 (sc-60329), ASC (sc-37282), and Caspase-1 (sc-29922) were purchased from Santa Cruz biotechnology.

Purification and Characterization of βγ-CAT. βγ-CAT was purified from the skin secretions of *B. maxima* as previously described (1). Briefly, lyophilized *B. maxima* skin secretions were separated with a DEAE Sephadex A-50 column at pH 7.3. The peak III of DEAE-Sephadex A-50 column was further separated with a Sephadex G-100 column. The peak II of Sephadex G-100 column was applied on an AKTA Mono-Q HR5/5 anion ion exchange column and resulted in separation of several protein peaks, and peak I was purified βγ-CAT. The purity of βγ-CAT was analyzed with native polyacrylamide gel electrophoresis (PAGE) or SDS/PAGE with silver staining, as shown in Fig. S5.

Cell Lines. Frog (*B. maxima*) peritoneal cells were used for in vitro experiments. The procedure for isolating frog peritoneal cells was as previously described (3). Mouse bone marrow-derived macrophages (BMDMs) were isolated from the femurs of mice and cultured as described by Riteau, et al. (4).

Bacterial Strains for Infection. To infect frogs, bacteria were isolated from the skin of live wild frogs. We collected *B. maxima* postmetamorphic individuals during the rainy seasons of 2011 and 2012 in Chuxiong, Yunnan Province, China. Before sampling, each individual was rinsed three times with 100 mL of sterile water to remove transient bacteria. Bacteria from frog skin were sampled using sterile cotton-tipped swabs and cultured onto a Standard I nutrient medium (Merck) at 30 °C for up to 72 h. DNA from these bacteria was then extracted from pure cultures with a TIANamp Bacteria DNA Kit and then amplified by PCR using universal bacterial 8F and 1492R 16SrRNA primers (8F, AGAGTTTGATCMTGGCTCAG; 1492R, GGYTACCTTGTTACGACTT). The products were sent to BGI Shenzhen for sequencing and were identified according to similarity with GenBank (www.ncbi.nlm.nih.gov/) and the Ribosomal Database Project (RDP-II) (http://rdp.cme.msu.edu/taxomatic/main.spr). The results are shown in Table S2. A *Comamonas* sp. strain was used for all frog infections.

To infect mice, bacterial strains were obtained from the First Affiliated Hospital of Kunming Medical University (China). Two different species were used: (*i*) *Escherichia coli* ATCC 25922 and (*ii*) *Staphylococcus aureus* ATCC 25923.

Bioinformatic Analysis. Protein domain searches were performed by using Motif Scan (http://hits.isb-sib.ch/cgi-bin/PFSCAN) software. The schematic structure of βγ-CAT was drawn with the DOG 2.0 program (5). Multiple alignments of the amino acid sequences of aerolysin domains were done with Vector NTI 11.5. The aligned sequences are vertebrate-derived aerolysin domain-containing proteins (Fig. S1*A*). Prediction of βγ-CAT aerolysin domain 3D structure was performed by using the I-Tasser server (6, 7). The 3D structure was obtained by multiple threading using the I-Tasser server (8). A confidence score (C-score) was used to estimate the quality of the computed model. A high C-score means a model with high confidence, which is often in the range of −5 to 2. And a C-score greater than −1.5 means a prediction of correct folding. The 3D structure of βγ-CAT aerolysin domain was compared and presented with aerolysin-like proteins with the software PyMOL.

Tissue Samples, RNA Extraction, and RT-PCR. Frog tissues were sampled from intact adult frogs. Total RNAs of these tissues were purified by using an RNA Easy kit (QIAGEN) according to the manufacturer's instructions. RNA yields and quality were checked by using agarose gel electrophoresis and a spectrophotometer (Thermo). Equal amounts of total RNA were used to synthesize the cDNA (TaKaRa) according to the protocol. The synthesized first cDNA strand was used as the template for semiquantitative or quantitative PCR. The primers used are listed in Table S4.

In Vivo Frog Experiments. Bacteria or βγ-CAT in 100 μL of 0.9% NaCl was injected into the peritoneum of the frog. At the indicated time, the frog was anesthetized with diethyl ether and then intraperitoneally injected (sterile syringe with a 20-G needle) with 2 mL of 0.9% NaCl prewarmed to 22 °C. The frog was then gently massaged, and the injected solution was collected into a sterile tube. The harvested peritoneal lavage fluids were used for bacterial counting. Cells were separated by centrifugation at 600 × *g* for 5 min for flow-cytometry analysis. Supernatants were obtained by centrifugation at 10,000 × *g* for 10 min and then dried by lyophilization and reconstituted to 100 μL. The suspension was used for Western blotting analysis.

For bacterial counting, the peritoneal lavage fluids were serially diluted by using the 10-fold dilution technique. Using the spread-plate method, 100 μL of each dilution was plated out in duplicate sets of standard I nutrient medium. The plates were then incubated at 30 °C for 18 h, and the distinct colonies that developed were counted. The bacterial numbers were expressed as log colony-forming units (cfu/mL). The same procedure was performed for the control.

To identify frog peritoneal exudate cells (PECs) by flow cytometry, PECs were collected by peritoneal lavage after 4 h, as described above in the first paragraph. Propidium iodide (Sigma) was added at a concentration of 1 μg/mL to exclude dead cells and debris. Flow-cytometry analysis was based on forward and side scatter on a FACSCalibur flow cytometer (Becton Dickinson) as previously reported for zebrafish (9). Wright's stain was also used to identify the PEC type that exhibited the greatest change.

Stimulation of Frog *B. maxima* Peritoneal Cells in Vitro. Frog (*B. maxima*) peritoneal cells were isolated as described above in the section *In Vivo Frog Experiments*. For short-term culture, the cells were cultured in DMEM/F12 (HyClone) supplemented with 10% FBS (HyClone). Peritoneal cells acquired from more than 30 frogs were mixed to perform all of the in vitro experiments. The cells (4×10^5 cells per mL) were primed with LPS (100 ng/mL) for 2 h, washed with PBS twice, and then cultured in the presence or absence of various agents assayed (βγ-CAT or pharmacological inhibitors) for the indicated time period. Finally, the cells were lysed for Western blotting, as previously described (10), and the supernatants were concentrated to 1/20th of the original volume for Western blotting analysis.

Western Blotting. Prepared protein samples were electrophoresed in an SDS-polyacrylamide gel and transferred onto a PVDF membrane. The membrane was subsequently blocked with 3% BSA and incubated with the appropriate primary and secondary antibodies. Protein bands were visualized with SuperSignal chemiluminescence reagents (Pierce), as previously described (11). Bands were semiquantified with ImageJ software. For data presented as bar graphs, control samples are set as 100%, and percent of change in intensity is reported. Data are representative of at least two to three independent experiments.

Native-PAGE and Western Blotting Detection of βγ-CAT. The native-PAGE–based Western blotting was carried out as described (12). The frog concentrated peritoneal fluids and purified βγ-CAT were separated by native-PAGE in a 10% gel. The native gel was soaked in 10% SDS for 5 min before transfer to a PVDF membrane (Millipore), and conventional Western blotting was subsequently carried out as described above in the section *Western Blotting*. The results were semiquantified with ImageJ and are shown in Fig. 1*E* and Fig. S1*B*.

Induction of Peritonitis in Mice. Kunming mice were purchased from the Animal Experimental Center of Kunming Medical University. Male mice weighing 18–22 g and aged 4 wk were used in all experiments. Generally, the experimental groups were composed of at least six mice. Animals from the same group were housed in individual cages at a constant temperature (22 °C) and humidity and with a 12-h light/dark cycle. These mice had access to food and water ad libitum throughout the study. The mice were euthanized by CO_2 inhalation at the end of the experiments.

Peritonitis was induced as described by Renckens et al. (13). In brief, bacteria were cultured in Luria–Bertani medium at 37 °C, harvested at midlog phase, and washed twice with sterile saline before injection. Mice were injected intraperitoneally with the appropriate amount of bacteria in 150 μL of sterile saline. The mortality observation was made every 12 or 24 h. For assessing the influences of βγ-CAT, it was injected 4 h before injection of the bacteria. A caspase-1 inhibitor Ac-YVAD-cmk was injected 1 h before βγ-CAT injection.

Mice Peritoneal Lavage Fluid Sampling, Cytokine Determination, and Bacteria Counting. At the time of euthanization, the abdomen of mice was first anesthetized by 75% ethanol. The peritoneal lavage was then harvested with 2 mL of sterile PBS using an 18-gauge needle, and the peritoneal lavage fluid was collected in sterile tubes. The collected peritoneal lavage fluid was directly used for bacterial counting. After centrifugation, the supernatant was used to assess the concentration of cytokine level, and the cells proceeded to flow-cytometry analysis.

For assessing the levels of IL-1β, KC (CXCL1), and MIP-2 (CXCL2), culture supernatants or peritoneal lavage fluids were centrifuged at $600 \times g$ for 10 min. Then, the cytokine concentration was determined by a commercial ELISA kit (R&D Systems) according to the manufacturer's instructions.

The peritoneal lavage was harvested and collected in sterile tubes, and eight serial 10-fold dilutions were made of each sample of the homogenates. Then, 100 μL of each dilution was plated onto blood agar plates. The plates were incubated at 37 °C for 20 h; then, the cfus were counted and corrected for the dilution factor. The threshold value of the method was 100 cfus.

Flow-Cytometry Analysis of Mice Peritoneal Exudate Cells. The PECs (peritoneal exudate cells) were washed with 1640 medium and blocked with 5% normal mouse serum for 10 min at room temperature. After the cells were washed with 1640 medium, FITC-conjugated anti–Gr-1 (Biolegend) and PE-conjugated anti-F4/80 (Biolegend) were added, and the cells were incubated at room temperature for 15 min. The cells were then analyzed on a FACS-Calibur flow cytometer (BD), and the Flowjo software was used to analyze the data.

Antimicrobial Assays. The direct amtimicrobial activity was assayed as previously described (14). Standard bacterial and fungal strains used in antimicrobial assays, Gram-positive bacterial strains *S. aureus* (ATCC2592) and *Bacillus megatherium,* Gram-negative bacterial strains *E. coli* (ATCC25922), *Bacillus pyocyaneus* (CMCCB10104), *Bacillus dysenteriae,* and *Klebsiella pneumoniae,* and fungal strains *Candida albicans* (ATCC2002), *Aspergillus flavus* (IFFI4015), and *Penicillium uticale* (IFFI2001) were obtained from Kunming Medical University. The bacteria were first grown in LB (Luria–Bertani) broth to an OD600 nm of 0.8. A 10-μL aliquot of the bacteria was then taken and added to 8 mL of fresh LB broth with 0.7% agar and poured over a 90-mm Petri dish containing 25 mL of 1.5% agar in LB broth. After the top agar hardened, a 20-μL aliquot of the sample filtered on a 0.22-μm Millipore filter was dropped onto the surface of the top agar and completely dried before incubation overnight at 37 °C. If an examined sample contained antimicrobial activity, a clear zone formed on the surface of the top agar representing killing the bacteria and/or inhibition of bacterial growth. Minimal inhibitory concentration (MIC) was determined in liquid LB medium by incubating the bacteria in LB broth with variable amounts of the sample tested. The MIC at which no visible growth occurred was recorded. In the assays of antifungi activity, for strains of *A. flavus* and *P. uticale,* the fungal-spore concentration was first counted under a microscope. The fungi with an initial concentration of 10^5 spores per mL were cultured with variable amounts of the sample tested in yeast extract-peptone-dextrose broth. The results are shown in Table S3.

Confocal Microscopy. For staining of Cathepsin B, Lamp-1, and βγ-CAT, cells cultured on glass coverslips for 24 h were treated with or without βγ-CAT. After being washed three times with ice-cold PBS, the cells were fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.05% Nonidet P-40 for 20 min at room temperature, and blocked with 1% BSA in PBS. The cells were subsequently incubated with primary antibodies overnight at 4 °C. The cells were then washed three times with PBS and incubated with FITC-labeled or Cy3-labeled secondary antibodies for 30 min at room temperature. The cells were washed three times, stained, and observed under the confocal microscope. Images were acquired using a confocal system (LSM 510 Image Examiner; Zeiss).

For AO (acridine orange) staining, cells were treated with or without βγ-CAT for 30 min; then, the cells were incubated with AO (0.5 μg/mL) and then visualized using a confocal system (LSM 510 Image Examiner; Zeiss). Quantification of fluorescence was determined in five random fields using ImageJ software.

Lysosome Preparation. Cytosol proteins and lysosomal proteins were prepared from frog peritoneal cells by using a lysosome isolation kit (Sigma-Aldrich). Cells treated with or without βγ-CAT

were washed with PBS twice. Then, the cells were resuspended in a 2.7 packed cell volume of extraction buffer. They were broken in a 7 mL Dounce homogenizer for 25 strokes and centrifuged at 1,000 × *g* for 10 min to discard the precipitated nucleus and unbroken cells. The supernatants were centrifuged at 20,000 × *g* for 20 min. The supernatants are the proteins from cytosol. The precipitated pellets, which represent the lysosome, were resuspended in 100 μL of extraction buffer. The cytosol and lysosome proteins were processed for detection of βγ-CAT or cathepsin B by Western blotting.

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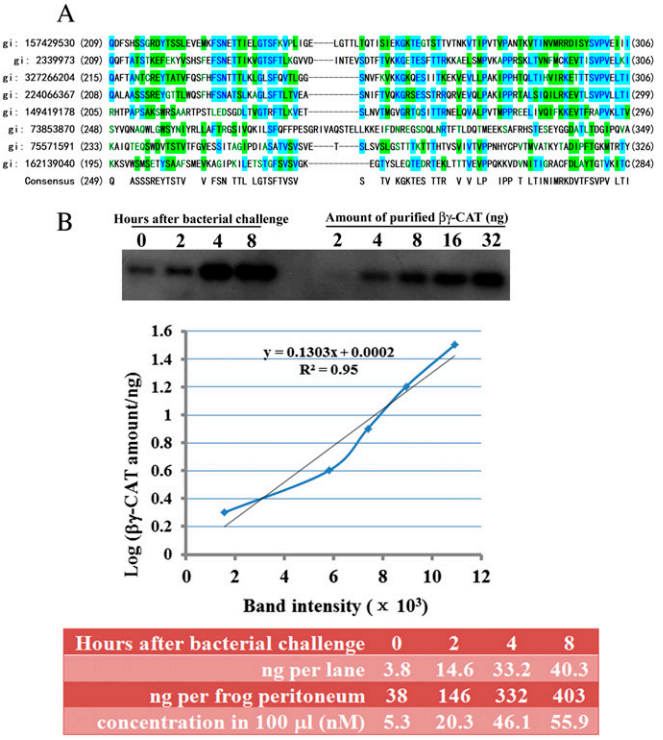

Hours after bacterial challenge	0	2	4	8
ng per lane	3.8	14.6	33.2	40.3
ng per frog peritoneum	38	146	332	403
concentration in 100 μl (nM)	5.3	20.3	46.1	55.9

Fig. S1. Related to Fig. 1. (*A*) Multiple sequence alignment of the conserved aerolysin domains of eight selected members of the vertebrate-derived aerolysin domain-containing proteins: βγ-CAT [GenInfo Identifier (gi): 157429530] from *B. maxima*, ep37-L2 (gi: 2339973) from *Cynops pyrrhogaster*, hypothetical protein LOC613112 (gi: 73853870) from *Xenopus (Silurana) tropicalis*, epidermal differentiation-specific protein-like protein (gi: 327266204) from *Anolis carolinensis*, glutamine-tRNA ligase (gi: 224066367) from *Taeniopygia guttata*, and natterin-3 precursor (gi: 75571591) from *Thalassophryne nattereri*, similar to ep37-L2 protein (gi: 149419178) from *Ornithorhynchus anatinus* and uncharacterized protein LOC568775 (gi: 162139040) from *Danio rerio*. (*B*) Semiquantification of βγ-CAT in frog peritoneum after bacterial challenge. The proteins of the concentrated frog peritoneal fluids were electrophoretically separated in a polyacrylamide gel (native-PAGE), and different amounts of purified βγ-CAT were loaded in the same gel. Then, the proteins were detected by Western blotting with rabbit polyclonal antibodies against βγ-CAT. The band intensity of purified βγ-CAT (right lanes) was calculated with ImageJ, and a standard curve was drawn. The band intensity of concentrated frog peritoneal fluids (left lanes) was calculated, and the amount of βγ-CAT produced in the frog peritoneal fluids was estimated by the standard curve. The results were repeated three times and the results are shown in Fig. 1*E*, *Lower*.

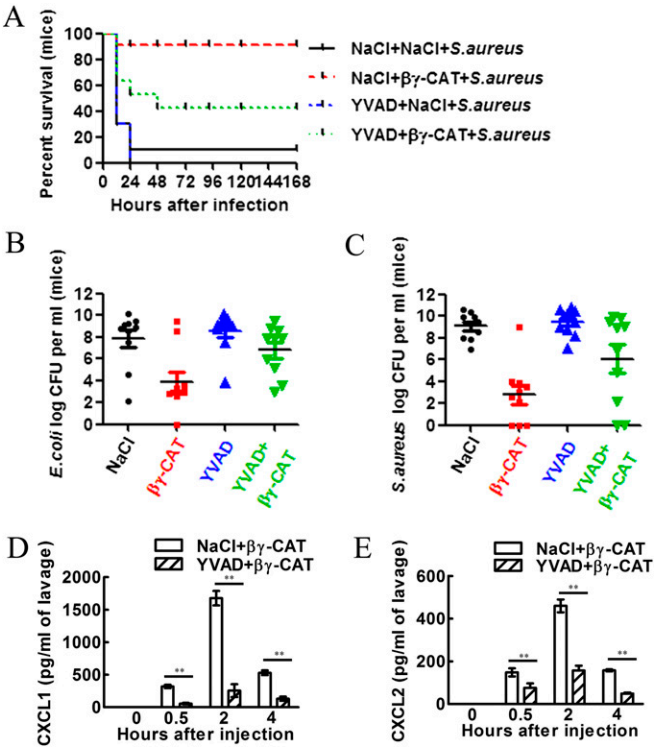

Fig. S2. Related to Fig. 2. βγ-CAT protects mice against bacterial infection. (*A*) Four-week-old male Kunming mice were intraperitoneally infected with *S. aureus* (ATCC 25923). Mortality of the mice was observed every 12 h; 25 μg/kg of βγ-CAT was intraperitoneally injected to assess its role in mice mortality. A caspase-1 inhibitor Ac-YVAD-cmk (5 mg/kg) was injected 1 h before βγ-CAT injection to assess the role of caspase-1 and inflammasome on mice mortality. (*B* and *C*) Four-week-old male Kunming mice were intraperitoneally infected with *E .coli* (ATCC 25922) (*B*) or *S. aureus* (ATCC 25923) (*C*), respectively. Bacteria counts were assayed 12 h after infection. βγ-CAT (25 μg/kg) was intraperitoneally injected before infection to assess its role in clearance of the bacteria. The caspase-1 inhibitor Ac-YVAD-cmk (5 mg/kg) was injected 1 h before βγ-CAT injection to assess the role of caspase-1. (*D* and *E*) Mice were injected intraperitoneally with βγ-CAT (25 μg/kg); then, the concentration of CXCL1 (*D*) and the concentration of CXCL2 (*E*) at indicated time points in mice peritoneum exudate were assessed by ELISA. The chemokines produced in the presence of Ac-YVAD-cmk (5 mg/kg) were also assayed. The survival rate and bacterial count data are representative of two experiments, with $n = 10$ mice per group. The ELISA data represent the mean ± SD of triplicate samples. $^{**}P < 0.01$ by Student *t* test.

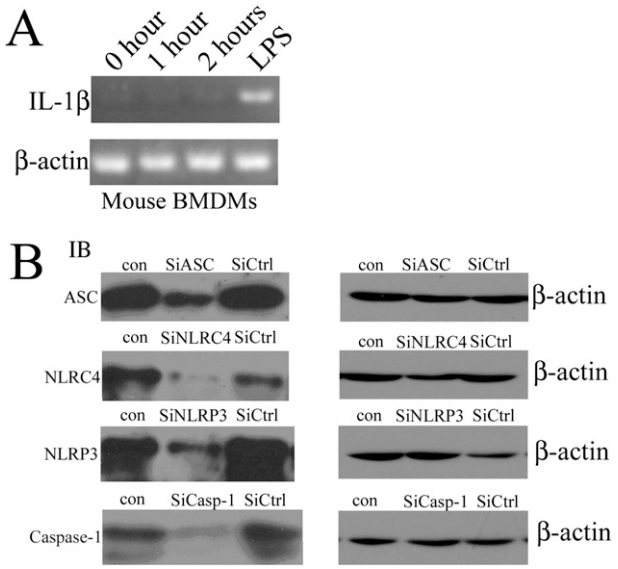

Fig. S3. Related to Fig. 3. (*A*) Mouse BMDMs were stimulated with βγ-CAT (5 nM) or LPS (100 ng/ml). Then, the expression of IL-1β was assessed by RT-PCR. (*B*) The ASC, NLRC4, NLRP3, or caspase-1 of mouse BMDMs was knocked down by RNA interference. The knockdown efficiency was determined by Western blotting.

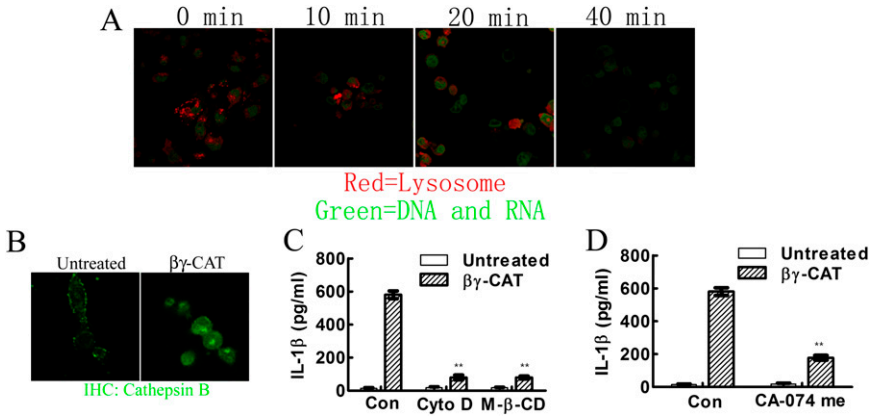

Fig. S4. Related to Fig. 4. βγ-CAT endocytosis and lysosome destabilization induce IL-1β secretion in mouse BMDMs. (*A*) βγ-CAT–treated (5 nM for 30 min) or untreated mouse BMDMs were stained with acridine orange (AO). Intact lysosomes could be visualized under a Zeiss LSM510 microscope (red). Fluorescence intensity may be attenuated when the lysosome was destabilized. βγ-CAT treatment obviously attenuated fluorescence intensity. (*B*) βγ-CAT–treated (5 nM for 30 min) or untreated mouse BMDMs were fixed and stained with a cathepsin B antibody. βγ-CAT treatment induced the leakage of cathepsin B from lysosome to cytosol. (*C*) Mouse BMDMs were pretreated with cytochalasin D (2 μM) or M-beta-CD (1 mM, a lipid raft disrupter) for 30 min; then, the cells were incubated with βγ-CAT (5 nM) for 2 h. The IL-1β concentrations in the supernatants were assessed by ELISA. (*D*) Cathepsin B inhibitor CA074-Me (30 μM) inhibited βγ-CAT–induced IL-1β secretion by mouse BMDMs. The ELISA data represent the mean ± SD of triplicate samples. $^{**}P < 0.01$ by Student *t* test.

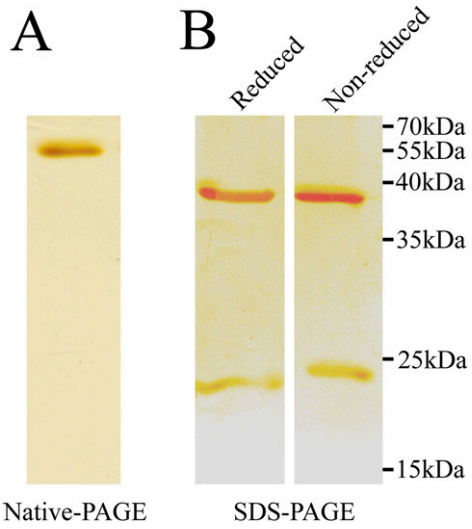

Fig. S5. Analysis of the purity of purified βγ-CAT preparation by native-PAGE and SDS/PAGE. (*A*) PAGE analysis of purified βγ-CAT (10 μg) under native conditions at pH 8.5 (10% acrylamide). (*B*) SDS/PAGE (10% acrylamide) analysis of purified βγ-CAT (10 μg) under reducing conditions (*Left*) and nonreducing conditions (*Right*). The protein bands were silver-stained.

Table S1. Proteins from vertebrates that share the same domain architecture with βγ-CAT α-subunit

Class	Species	Annotation	Gi no.
Fish	*Clarias batrachus* (walking catfish)	Epidermal differentiation-specific protein	529164852
Amphibians	*Bombina maxima* (large-webbed belly toad)	Ebetagamma-cat alpha subunit	157429530
	Cynops pyrrhogaster (Japanese firebelly newt)	ep37-L2	2339973
		Epidermal differentiation-specific protein	286130
		ep37-A1	2339969
		ep37-A2	2339971
		gep	6069419
Reptiles	*Anolis carolinensis* (green anole)	Epidermal differentiation-specific protein-like	327265713
	Anolis carolinensis (green anole)	Epidermal differentiation-specific protein-like	327266204
	Chrysemys picta bellii (western painted turtle)	Epidermal differentiation-specific protein-like	530608856
Birds	*Melopsittacus undulatus* (budgerigar)	Epidermal differentiation-specific protein-like	527246552
	Zonotrichia albicollis (white-throated sparrow)	Epidermal differentiation-specific protein-like	542159345
	Ficedula albicollis (collared flycatcher)	Epidermal differentiation-specific protein-like	525011286
	Geospiza fortis (medium ground-finch)	Epidermal differentiation-specific protein-like	543257987
	Falco cherrug (Saker falcon)	Epidermal differentiation-specific protein-like	541952063
	Pseudopodoces humilis (Tibetan ground-tit)	Epidermal differentiation-specific protein-like	543355479
	Columba livia (rock pigeon)	Epidermal differentiation-specific protein-like, partial	543733246

The proteins sharing the same domain architecture with βγ-CAT were found by protein blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The Gi numbers and annotations of these proteins are listed in this table.

Table S2. List of bacterial groups identified from the skin of *Bombina maxima*

Bacterial groups	Phylum	Class	Order	Family	Genus
1	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Comamonas*
2	*Firmicutes*	*Bacilli*	*Bacillales*	*Staphylococcaceae*	*Staphylococcus*
3	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Alcaligenaceae*	—
4	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	*Stenotrophomonas*

The bacteria on the frog (*B. maxima*) skin were cultured and characterized as described in *SI Materials and Methods*.

Table S3. Direct antimicrobial activity assay

	MIC, μg/mL	
Microorganisms	Maximin 3	βγ-CAT
Escherichia coli ATCC 25922	0.9	ND
Staphylococcus aureus ATCC25923	3.1	ND
Bacillus pyocyaneus CMCCB10104	1.5	ND
Bacillus megatherium	0.8	ND
Bacillus dysenteriae	0.9	ND
Klebsiella pneumoniae	3.2	ND
Candida albicans ATCC2002	15	ND
Aspergillus flavus IFFI4015	ND	ND
Penicillum uticale IFFI2001	ND	ND
Comamonas sp	ND	ND

Direct antimicrobial effects were tested as described in *SI Materials and Methods*. Maximin 3 is an antimicrobial peptide purified from the skin of the frog *B. maxima* (14). MIC, minimal inhibitory concentration; ND, not detectable. The direct antimicrobial effect of βγ-CAT cannot be detected even at a concentration as high as 50 μg/mL.

Table S4. Sequences of Primers used for detection of βγ-CAT and IL-1β

Primer	Sequence	Cycles	Product length
Bombina maxima			
βγ-CAT-α-Forward	GCTTCCTCTCTGCGTGTGAT	28	169
βγ-CAT-α-Reverse	GCTTGATAACTGGGTCCCCC		
βγ-CAT-β-Forward	GGCTGTGGCCATGGATTTTC	28	112
βγ-CAT-β-Reverse	GCCTGGCATTGCATTGTTCT		
β-actin-Forward	GTAGCCCCTGAAGAACACCC	25	238
β-actin-Reverse	TTGCATGGGGCAGAGCATAA		
IL-1β-Forward	AGATCTACACTGGGGCTCCT	30	307
IL-1β-Reverse	TTCTTGCAAGGCTAGGCACT		
Mouse			
IL-1β-Forward	GCTACCTGTGTCTTTCCCGT	30	164
IL-1β-Reverse	CATCTCGGAGCCTGTAGTGC		
β-actin-Forward	CCACCATGTACCCAGGCATT	25	253
β-actin-Reverse	AGGGTGTAAAACGCAGCTCA		