

Supplementary material

Table S1. NKL sequences used for phylogenetic analysis and sequence alignment in this study.

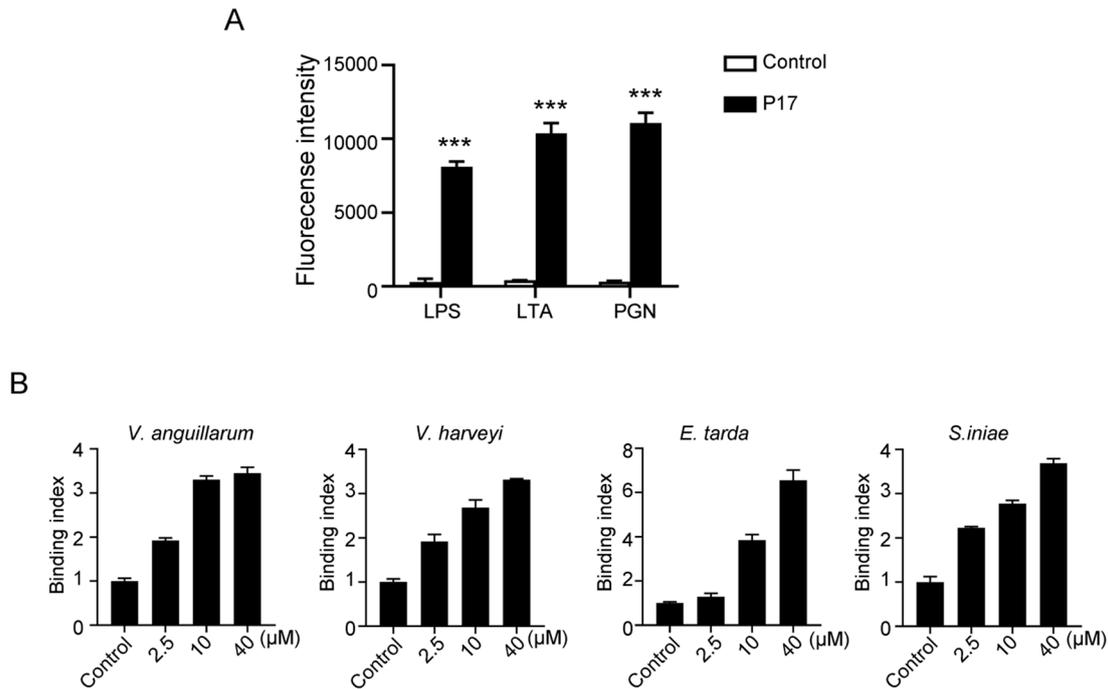
Accession number	Species	Protein
XM_020919797	<i>Boleophthalmus pectinirostris</i>	NKL
AGM21637	<i>Cynoglossus semilaevis</i>	NKL
XM_026230159	<i>Carassius auratus</i>	NKL
KT877168	<i>Ctenopharyngodon idella</i>	NKL
NM_001303323	<i>Larimichthys crocea</i>	NKL
MF113051	<i>Lateolabrax japonicus</i>	NKL
XM_018663395	<i>Latescal carifer</i>	NKL
XM_003962706	<i>Takifugu rubripes</i>	NKL
KU705506	<i>Scophthalmus maximus</i>	NKL
XM_015969930	<i>Nothobranchius furzeri</i>	NKL
MF678822	<i>Oreochromis niloticus</i>	NKL
XM_020487202	<i>Oncorhynchus kisutch</i>	NKL
XM_014129907	<i>Salmo salar</i>	NKL
XM_021574735	<i>Oncorhynchus mykiss</i>	NKL
XM_004082250	<i>Oryzias latipes</i>	NKL
XM_021473084	<i>Danio rerio</i>	NKL
KP100115	<i>Danio rerio</i>	NKLa
KP100116	<i>Danio rerio</i>	NKLb
KP100117	<i>Danio rerio</i>	NKLc
KP100118	<i>Danio rerio</i>	NKLd
NM_001200208	<i>Ictalurus punctatus</i>	NKL1
DQ153186	<i>Ictalurus punctatus</i>	NKL2
DQ153187	<i>Ictalurus punctatus</i>	NKL3
AU260449	<i>Paralichthys olivaceus</i>	NKL
XP_035024225.2	<i>Hippoglossus stenolepis</i>	NKL
XP_019940439.1	<i>Paralichthys olivaceus</i>	PREDICTED: prosaposin-like
KAF5165287.1	<i>Hippoglossus stenolepis</i>	Hypothetical protein
XP_041669156.1	<i>Cheilinus undulatus</i>	Antimicrobial peptide NK-lysin
XP_018518922.1	<i>Lates calcarifer</i>	PREDICTED: saposin-C-like
XP_035529581.1	<i>Morone saxatilis</i>	NK-lysin-like
ALH22547.1	<i>Larimichthys crocea</i>	NK-lysin-like type 2 protein
XP_005477177.1	<i>Oreochromis niloticus</i>	Antimicrobial peptide NK-lysin

Table S2. Primers used in this study.

Primer	Sequence(5'→3')
RT-β-Actin-F	AACCGCTGCCTCCTCCTCAT
RT-β-Actin-R	TCGGGACAACGGAACCTCTC
RT-NKLnc-F	TGCGGAATGGAGCATTTCCT
RT-NKLnc-R	TCCATTGCAGGCCTTGTTCA
RT-18S rRNA-F	GGTCTGTGATGCCCTTAGATGTC
RT-18S rRNA-R	AGTGGGGTTCAGCGGGTTAC
RT-α-tubulin-F	TGACATCACAAACGCCTGCTTC
RT-α-tubulin-R	GCACCACATCTCCACGGTACAG
RT-GAPDH-F	CAACGGCGACACTCACTCCTC
RT-GAPDH-R	TCGCAGACACGGTTGCTGTAG
NKLnc-F	ATGAGTCCTGTTTTTCAGCATCG
NKLnc-R	CTTTTTGCAGAGTTTCGCTTT

Table S3. The sequences of the siRNAs used for gene knockdown.

SiRNA	Forward primer (5'→3')	Reverse primer (5'→3')	Target gene
siR1	GGAACAAACAGAAGAGGAUTT	AUCCUCUUCUGUUUGUUCCTT	NKLnc
siR2	CCCACCAUGAGAAUCCCAATT	UUGGGAUUCUCAUGGUGGGTT	NKLnc
NCS	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT	None

**Figure S1. Bacteria binding activity of P17.** (A) The binding of P17 to different bacterial cell wall components was determined by ELISA. Values are the means of triplicate experiments and shown as means ± SD. *** $P < 0.001$. (B) Binding of P17 in different doses to bacteria *Vibrio anguillarum*, *Vibrio harveyi*, *Edwardsiella tarda* and *Streptococcus iniae* was determined by ELISA.

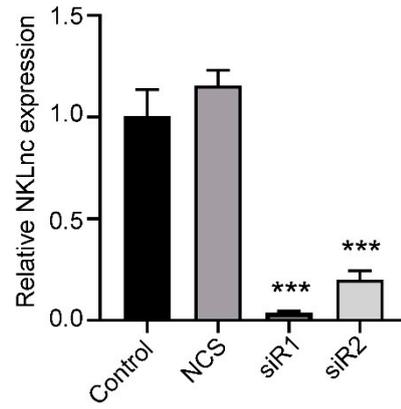


Figure S2. Verification of NKLnc knockdown. (A) FG-9307 cells were treated with or without (control) siR1, siR2, or the negative control siRNA (NCS) for 24 h, and NKLnc expression was determined by RT-qPCR. Values are the means of triplicate experiments and shown as means \pm SD. *** P < 0.001.