

Supplementary Materials

A novel proline-rich cathelicidin from the alpaca *Vicugna pacos* with potency to combat antibiotic-resistant bacteria: mechanism of action and the functional role of the C-terminal region

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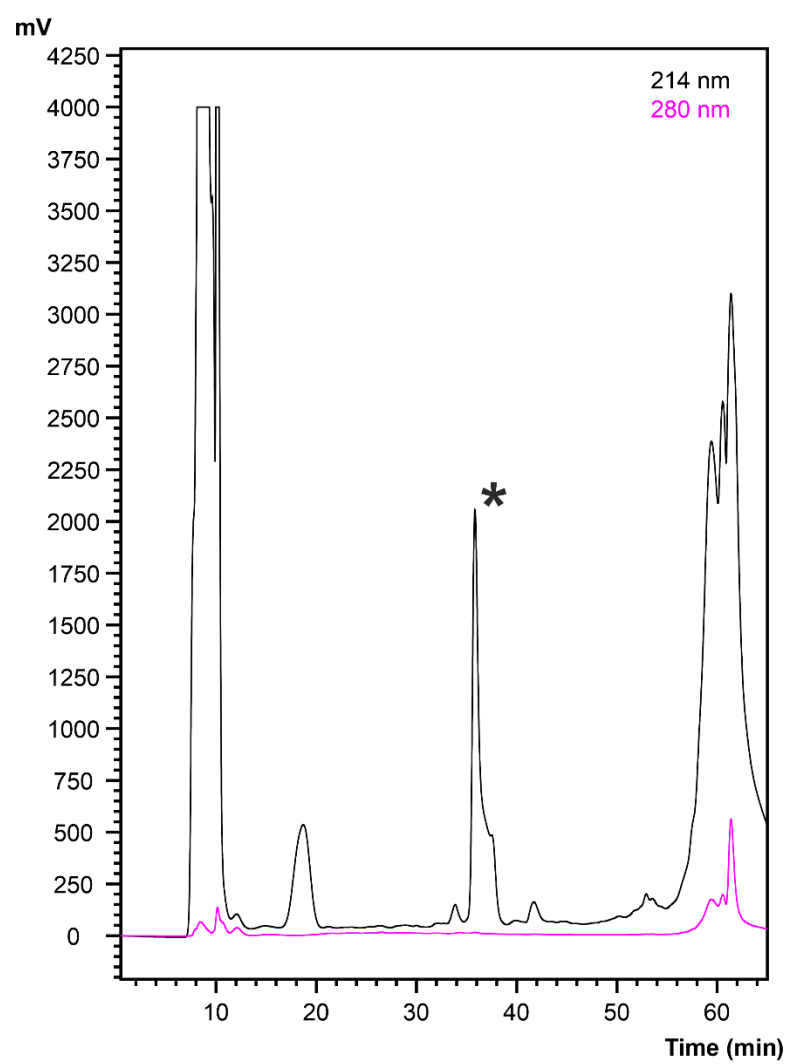


Figure S1. Reverse-phase high-performance liquid chromatography (RP-HPLC) purification of the recombinant VicBac[1-22]. The fraction of the target peptide is marked with an asterisk.

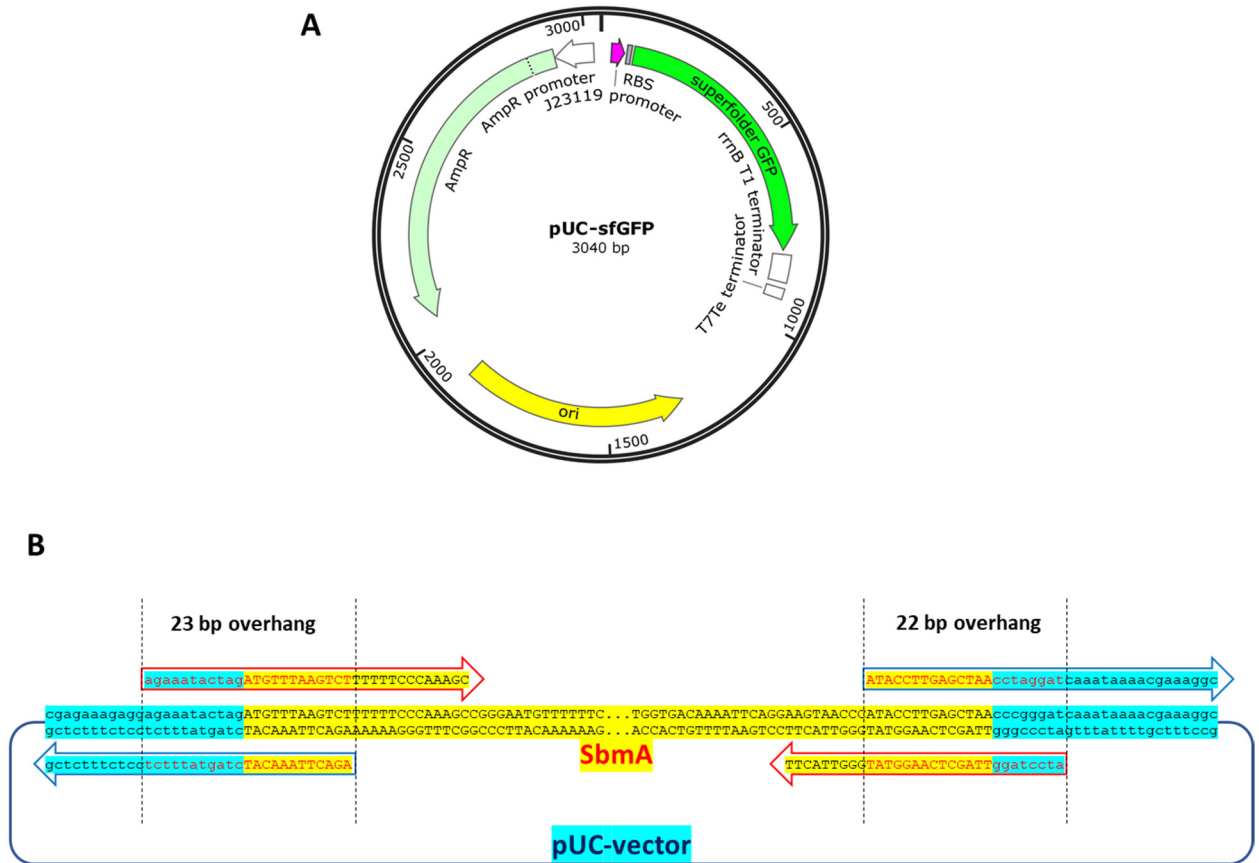


Figure S2. (A) Plasmid vector for the expression of sfGFP under the strong constitutive artificial promoter J23119. Map was visualized with SnapGene software. **(B)** The scheme of DNA-amplification, ligase-independent assembly of the complementation plasmids overexpressing SbmA variants in SbmA-deficient *E. coli*.

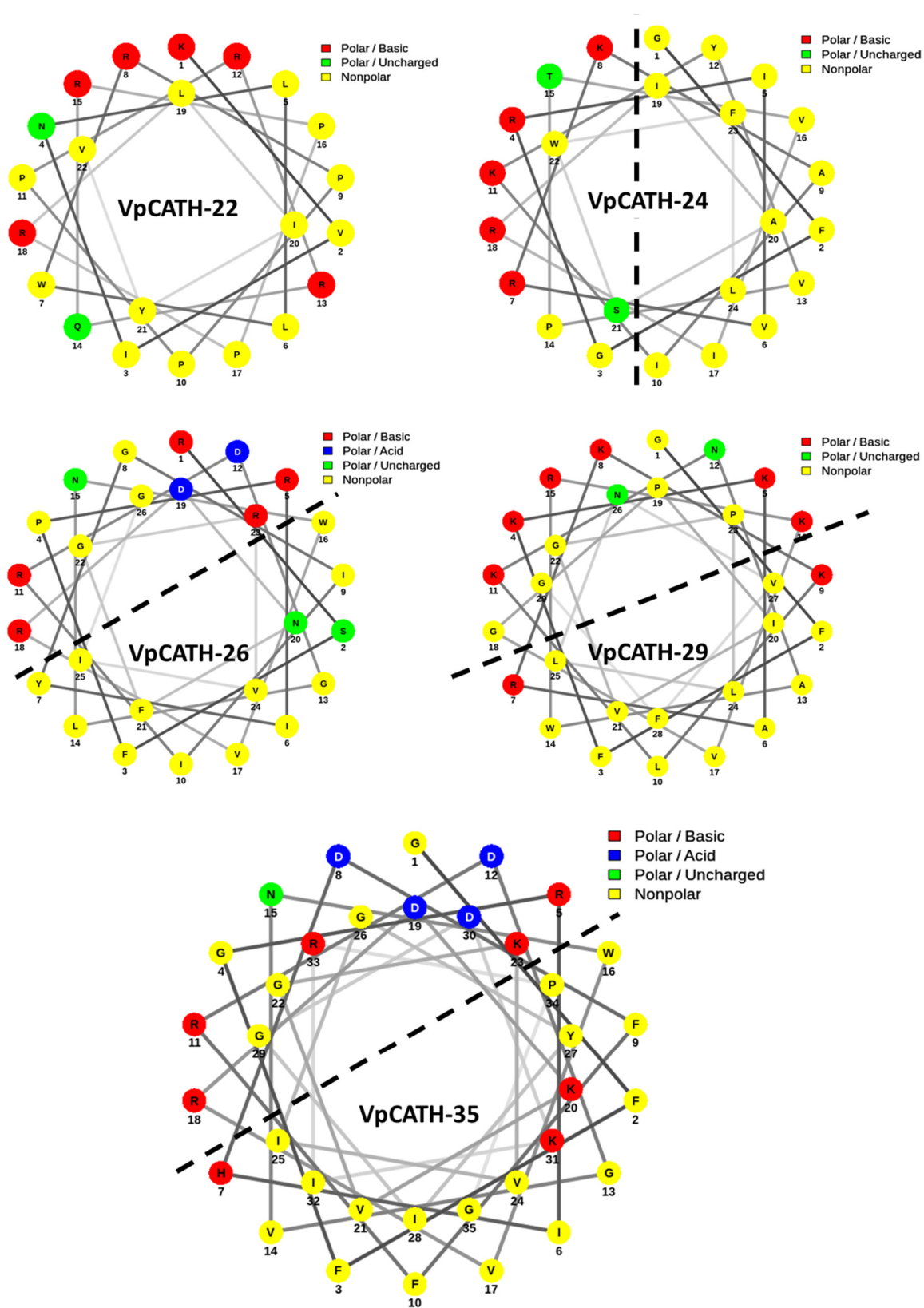


Figure S3. Helical wheel projections of alpaca cathelicidins. The projections were generated by online NetWheels tool (<http://lbqp.unb.br/NetWheels/>).

METQRASLSLRWSLWLLLLGLAVPWASA
QALSYREAVLRAVDRLNEQSSDPNLYRLLLELDLPKADEDPDAPKPVSFYTKETV
CPRRTQLPQEQCA
FKENGVVVK
CLGTVNLYQLRDNFDIT
CNELOSQV
KVINLLWRPPRRRPPRLIYV
(VpCATH-22)

Vicugna pacos (JEMW01020494.1)

METQRASLSLRCSWLWLLLLGLAVPWASAQALSYREAVLRAVDRLNEQSSDPNLYRLLLELDLPPKADENPDAPKPVSMVMKETVCPRRTQQPPEQCD
 FKENGVVVKQCLGIVTLDOITGOLAVTCDELOSV**GFGRIVRKAIKYVPTVIRIASWFL** (VpCATH-24)

[illegible]

Figure S4 (part 1). Structure of *CATHL* genes and corresponding preprocathecidins of *Vicugna pacos*. Predicted intron/exon boundaries are highlighted in red. Exons are highlighted in green. Signal peptide sequences are marked in yellow/black. Signal peptide sequence identified with SignalP 5.0 (www.cbs.dtu.dk/services/SignalP/). Peptide sequences of putative mature cathelicidins are marked with red font. The TATA-box signal is in bold and grey marked and the polyadenylation signal is in italics and grey marked.

Vicugna pacos (GenBank: ABRR03000981.1)

METQRDSLSLRWSLWLLLLGLAVPWASAQALSYREAVLRAVDRLNEQSSDPNLYRLLLELDLPKADEDPDAPKPVSFYTKETVCPRRQTQLPPQQCA
FKENGVVVKQCLGTVNLYQLRDNFDITCNELQSV**RSFPRIYGIIRDGLNWVRDNFGRVIG** (VpCATH-26)

ggagaggccaagaattttcaggagatggagaaacattttcttcaccttccacagggggcagacctcaccaccgcccagcaatccctgaggcaag
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Vicugna pacos (GenBank: ABRR03000981.1)

MDTQRASLSLRWSLWLLLLGLAVPWASIEALSYREAVLRAVDRLNEQSSDPNLYRLLLELDLPKAYEDLDAPKPVSFYTKETVCPRRQTQLPPEQCA
FKENGLVKQCVGTTTLDQVKQGMNIACDELQSI**GFFKKARKKLKNARWKVGPVIGPLLVFG** (VpCATH-29)

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ggcgtgtgtgtaactc

Figure S4 (part 2). Structure of *CATHL* genes and corresponding preprocathelelids of *Vicugna pacos*. Predicted intron/exon boundaries are highlighted in red. Exons are highlighted in green. Signal peptide sequences are marked in yellow/black. Signal peptide sequence identified with SignalP 5.0 (www.cbs.dtu.dk/services/SignalP/). Peptide sequences of putative mature cathelids are marked with red font. The TATA-box signal is in bold and grey marked and the polyadenylation signal is in italics and grey marked.

METQRDLSLRRLWLLLLGLAVPWASTEAELSYREAVLRAVDRLNEQSSDPNLYRLLLELDLPKKAEDLDAPKPVSFYVKETVCPRRITQLPPEQCA
 FKENGVVVKQLGTNLYQLRDNYDITCNELOSVGFFGRIHDFFRDGVNVVRDKVGKIVIGYIGDKIRPG (VpCATH-35)

METQRASLSLRRLWILLGLAVPWASAQALSYREAVLRAVDRLNEQSSDPNLYRLLLELDLPPKAEDLDAPKPVSFYTKETVCPRRQLPPEQCD
 FKEKGVVVKLCIGTVDLDQVDQDIDISCNKLQSV**RRFFPRFPFPGPFPPFPPIPPIPFPFPGPIIR**GRR (VpCATH-38)

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Figure S4 (part 3). Structure of *CATHL* genes and corresponding preprocathecidins of *Vicugna pacos*. Predicted intron/exon boundaries are highlighted in red. Exons are highlighted in green. Signal peptide sequences are marked in yellow/black. Signal peptide sequence identified with SignalP 5.0 (www.cbs.dtu.dk/services/SignalP/). Peptide sequences of putative mature cathelicidins are marked with red font. The TATA-box signal is in bold and grey marked and the polyadenylation signal is in italics and grey marked.

METQRDLSLSRRWSLWLLLLGLAVPWASA QALSYREAVLRAVDRLNEQSSDPNLYRLLLELDLPKKADEDPDAPKPVSFYVKETV CPKRTQQPPEQCA
 FKENGVVVKQCVGTVTLDSRDOODITCDELSQVRRIRRRPRLPRPRVPRPRIPPRIPRVLPPPPRVFPFPRFPRGR (VicBac)

Vicugna pacos (GenBank: ABRR03000981.1)

gcaatccccctgaggcaagagcggggccttaggcaagctccagctcacacccctggccgggggcagggatggggcaggtggggaaggctcctgcttgagc
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Figure S4 (part 4). Structure of *CATHL* genes and corresponding preprocathecidins of *Vicugna pacos*. Predicted intron/exon boundaries are highlighted in red. Exons are highlighted in green. Signal peptide sequences are marked in yellow/black. Signal peptide sequence identified with SignalP 5.0 (www.cbs.dtu.dk/services/SignalP/). Peptide sequences of putative mature cathelicidins are marked with red font. The TATA-box signal is in bold and grey marked and the polyadenylation signal is in italics and grey marked.

SbmA (wt)	ATGTTTAAAGTCTTTTTTCCCAAAGCCGGGAATGTTTTTCTCTCGGCCCTTGTGTTGGGCATTGATTGCCGTATCTTCTG	80
SbmA (mut)	ATGTTTAAAGTCTTTTTTCCCAAAGCCGGGAATGTTTTTCTCTCGGCCCTTGTGTTGGGCATTGATTGCCGTATCTTCTG	80
Gap fraction		
SbmA (wt)	GCAAGTCGGTGGGGTGATTGGGTGGCGCGTATCACCGGCGCTTCCGGGCAGATCCCGATTAGCGCCGCGCGTTTCTGGT	160
SbmA (mut)	GCAAGTCGGTGGGGTGATTGGGTGGCGCGTATCACCGGCGCTTCCGGGCAGATCCCGATTAGCGCCGCGCGTTTCTGGT	160
Gap fraction		
SbmA (wt)	CGTTGGATTTCTGATTTTTTACGCTTACTACATTGTTTGCCTAGGACTTTTTGCATTGTTCTGGTTTATCTATAGCCCG	240
SbmA (mut)	CGTTGGATTTCTGATTTTTTACGCTTACTACATTGTTTGCCTAGGACTTTTTGCATTGTTCTGGTTTATCTATAGCCCG	240
Gap fraction		
SbmA (wt)	CACCGTTGGCAATACTGGTCAATACTCGGTACTGCGCTGATTATCTTCGTACCTGGTTTTTGGTGAAGTCGGGGTTGC	320
SbmA (mut)	CACCGTTGGCAATACTGGTCAATACTCGGTACTGCGCTGATTATCTTCGTACCTGGTTTTTGGTGAAGTCGGGGTTGC	320
Gap fraction		
SbmA (wt)	CGTCAACGCCTGGTATGCGCCGTTCTATGATCTGATTCAAACGGCGCTAAGTTCGCCGCATAAAGTCACCATCGAACAAT	400
SbmA (mut)	CGTCAACGCCTGGTATGCGCCGTTCTATGATCTGATTCAAACGGCGCTAAGTTCGCCGCATAAAGTCACCATCGAACAAT	400
Gap fraction		
SbmA (wt)	TTTACC GCGAAGTGGCGCTCTTTCTGGGGATTGCGCTGATCGCGGTGGTGATCAGTGTGCTGAACAACCTCTTTGTCAGT	480
SbmA (mut)	TTTACC GCGAAGTGGCGCTCTTTCTGGGGATTGCGCTGATCGCGGTGGTGATCAGTGTGCTGAACAACCTCTTTGTCAGT	480
Gap fraction		
SbmA (wt)	CACTACGTGTTCCGCTGGCGTACGGCGATGAACGAATATTACATGGCGAACTGGCAACAACGCGTCATATCGAAGGGGC	560
SbmA (mut)	CACTACGTGTTCCGCTGGCGTACGGCGATGAACGAATATTACATGGCGAACTGGCAACAACGCGTCATATCGAAGGGGC	560
Gap fraction		
SbmA (wt)	CGCGCAGCGTGTGCAGGAAGACACCATGCGTTTTGCTTCAACGCTGGAGAATATGGGCGTCAGCTTTATCAACGCTATCA	640
SbmA (mut)	CGCGCAGCGTGTGCAGGAAGACACCATGCGTTTTGCTTCAACGCTGGAGAATATGGGCGTCAGCTTTATCAACGCTATCA	640
Gap fraction		
SbmA (wt)	TGACGTTGATCGCCTTCCTGCCGGTGCTGGTAACGCTCTCCGCACATGTGCCGGAGCTGCCGATTGTGGGCACATTCCG	720
SbmA (mut)	TGACGTTGATCGCCTTCCTGCCGGTGCTGGTAACGCTCTCCGCACATGTGCCGGAGCTGCCGATTGTGGGCACATTCCG	720
Gap fraction		
SbmA (wt)	TATGGTCTGGTGATTGCCGCTATCGTCTGGTCTGATGGGGACCGGATTACTGGCAGTGGTAGGGATCAAACGCGGG	800
SbmA (mut)	TATGGTCTGGTGATTGCCGCTATCGTCTGGTCTGATGGGGACCGGATTACTGGCAGTGGTAGGGATCAAACGCGGG	800
Gap fraction		
SbmA (wt)	GCTGGAGTTTAAAAACGAGCGTGTAGAGGCTGCCTACCGTAAAGAACTGGTTTATGGTGAAGACGATGCCACGCGCGTGA	880
SbmA (mut)	GCTGGAGTTTAAAAACGAGCGTGTAGAGGCTGCCTACCGTAAAGAACTGGTTTATGGTGAAGACGATGCCACGCGCGTGA	880
Gap fraction		
SbmA (wt)	CGCCGCCTACGGTACGCGAGCTTTTTAGCGCCGTACGGAAAACTATTTCCGCCTCTATTTTCTACTATATGTATTTCAAT	960
SbmA (mut)	CGCCGCCTACGGTACGCGAGCTTTTTAGCGCCGTACGGAAAACTATTTCCGCCTCTATTTTCTACTATATGTATTTCAAT	948
Gap fraction		
SbmA (wt)	ATCGCCCGCATTTCTCTATTTGCGAGGTCGATAACGTTTTCGGTTTGTCTTGCTGTTTCCGTCAATTGTTGCCGGTACGAT	1040
SbmA (mut)	ATCGCCCGCATTTCTCTATTTGCGAGGTCGATAACGTTTTCGGTTTGTCTTGCTGTTTCCGTCAATTGTTGCCGGTACGAT	1028
Gap fraction		
SbmA (wt)	TACACTCGGCCTGATGACGCAGATTACTAACGTTTTTGGTCAGGTTTCGCGGTGCTTTCCAGTACCTGATTAACATCATGA	1120
SbmA (mut)	TACACTCGGCCTGATGACGCAGATTACTAACGTTTTTGGTCAGGTTTCGCGGTGCTTTCCAGTACCTGATTAACATCATGA	1108
Gap fraction		
SbmA (wt)	CCACACTGGTTGAGTTGATGTCTATCTACAAACGCTGCGCAGCTTTGAACATGAGCTGGATGGTGACAAAATTCAGGAA	1200
SbmA (mut)	CCACACTGGTTGAGTTGATGTCTATCTACAAACGCTGCGCAGCTTTGAACATGAGCTGGATGGTGACAAAATTCAGGAA	1188
Gap fraction		
SbmA (wt)	GTAACCCATACCTTGAGCTAA	1221
SbmA (mut)	GTAACCCATACCTTGAGCTAA	1209
Gap fraction		

Figure S5. Alignment of nucleotide sequences of genes encoding SbmA from *E. coli* MDR CI 1057 "wt" (wild type) and mutant SbmA from VicBac[1-22]-resistant *E. coli* MDR CI 1057 "mut" obtained after selection. Nucleotide deletion is highlighted with red.

Table S1. Amino acid sequences and molecular masses of the peptides used in this study

Peptide	Origin	Sequence	Molecular mass, Da	
			Calculated [M+H] ⁺ value ¹	Measured value ²
Bac7[1-22]	Rec	RRIRPRPPRLPRPRPRPLPFPR	2783.72	2783.89
PR-39[1-22]	Rec	RRRPRPPYLPRPRPPFFFPRL	2765.61	2765.35
VicBac[1-14]	Rec	RRIRRPRLPRPRVP	1825.17	1824.96
VicBac[1-16]	Rec	RRIRRPRLPRPRVPRP	2078.32	2078.05
VicBac[1-18]	Rec	RRIRRPRLPRPRVPRPRI	2347.51	2347.25
VicBac[1-20]	Rec	RRIRRPRLPRPRVPRPRIPP	2541.61	2541.52
VicBac[1-22]	Rec	RRIRRPRLPRPRVPRPRIPPRI	2810.80	2810.84
VicBac[1-39]	Synt	RRIRRPRLPRPRVPRPRIPPRIPRPVLPFPRVPFPRFPR*	4815.98	4815.70
Melittin	Synt	GIGAVLKVLTTGLPALISWIKRKRQQ	2846.74	2846.60
LL-37	Synt	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES	4491.58	4491.40

Rec – recombinant peptide

Synt – synthetic peptide

* C-terminal amidation

¹ according to Expasy Isotopident tool

² monoisotopic m/z were measured using MALDI-TOF MS

Table S2. Amino acid sequences of natural cathelicidins from *Camelidae* species

Animal	Sequence	GenBank ID
Vp22-like cathelicidins		
<i>Camelus dromedarius</i>	KIINLPWRPPRRKRPIRVIYV	JWIN01013794.1 LSZX01138262.1
<i>Camelus ferus</i>	KIINLPWRPPRRKRPIRVIYV	AGVR01057826.1
<i>Camelus bactrianus</i>	KIINLPWRPPRRKRPIRVIYV	JARL01035148.1
<i>Vicugna pacos</i>	KVINLLWRPPRRQRPPRLIYV	JEMW01023459.1
<i>Vicugna vicugna</i>	KVINLLWRPPRRQRPPRLIYV	PNXW01017670.1 JEMW01026878.1
<i>Lama guanicoe</i>	KVINLLWRPPRRQRPPRLIYV	PNXV01010685.1
<i>Lama glama</i>	KVINLLWRPPRRQRPPRLIYV	PNXU01026596.1
23-residue cathelicidins (MAP-like)		
<i>Camelus dromedarius</i>	GLFGPILGIVRAGVKLVLDKFGK	JWIN01033265.1 JDVD01079641.1
<i>Camelus bactrianus</i>	GLFGPILGIVRAGVKLVLDKFGK	JARL01035147.1
<i>Lama guanicoe</i>	GILGPILRIVAAGLDWLQHKRGK	PNXV01004884.1
<i>Lama glama</i>	GILGPILRIVAAGLDWLQHKRGK	PNXU01036743.1
Vp24-like cathelicidins (MAP-like)		
<i>Camelus dromedarius</i>	GLGRIVRKAIKYVPTVIRIASWFL	LSZX01137296.1 JWIN01015089.1
<i>Camelus ferus</i>	GLGRIVRKAIKYVPTVIRIASWFL	AGVR01002349.1
<i>Camelus bactrianus</i>	GLGRIVRKAIKYVPTVIRIASWFL	JARL01005658.1
<i>Vicugna pacos</i>	GFGRIVRKAIKYVPTVIRIASWFL	ABRR02174663.1 JEMW01020494.1 ABRR02172633.1
<i>Vicugna vicugna</i>	GFGRIVRKAIKYVPTVIRIASWFL	PNXW01014781.1
<i>Lama guanicoe</i>	GFGRIVRKAIKYVPTVIRIASWFL	PNXV01005610.1
<i>Lama glama</i>	GFGRIVRKAIKYVPTVIRIASWFL	PNXU01002030.1
Vp26-like cathelicidins (MAP-like)		
<i>Vicugna pacos</i>	RSFPRIYGIIRDGLNWVRDNFGRVIG	JEMW01026878.1
<i>Vicugna vicugna</i>	RSFPRIYGIIRDGLNWVRDNFGRVIG	PNXW01021834.1
<i>Lama guanicoe</i>	RSFPRIFGIIRDGLNWVRDKLGRVIG	PNXV01022927.1
<i>Lama glama</i>	RSFPRIFGIIRDGLNWVRDKLGRVIG	PNXU01023268.1
Vp29-like cathelicidins (MAP-like)		
<i>Camelus dromedarius</i>	GFFKKARNKLKNAWRKVGPIVGPLLTFFG	JWIN01010394.1 LSZX01137298.1
<i>Camelus ferus</i>	GFFKKARNKLKNAWRKVGPIVGPLLTFFG	AGVR01002349.1
<i>Camelus bactrianus</i>	GFFKKARNKLKNAWRKVGPIVGPLLTFFG	JARL01005658.1
<i>Vicugna pacos</i>	GFFKKARKKLKNAWRKVGPIVGPLLNVFG	JEMW01020494.1 ABRR02172634.1
<i>Vicugna vicugna</i>	GFFKKARKKLKNAWRKVGPIVGPLLNVFG	PNXW01014781.1
<i>Lama guanicoe</i>	GFFKKARKKLKNAWRKVGPIVGPLLNVFG	PNXV01005610.1
<i>Lama glama</i>	GFFKKARKKLKNAWRKVGPIVGPLLNVFG	PNXU01002030.1
Vp38-like cathelicidins		
<i>Camelus dromedarius</i>	RGFFPPPPFPPGPFPPPPFPIPPFPPFPRPFVRRRR	JDVD01002168.1 JWIN01034589.1
<i>Camelus ferus</i>	RGFFPPPPFPPGPFPPPPFPIPPFPPFPRPFVRRRR	AGVR01053365.1
<i>Camelus bactrianus</i>	RGFFPPPPFPPGPFPPPPFPIPPFPPFPRPFVRRRR	JARL01035147.1
<i>Vicugna pacos</i>	RRFFPRPFPPGPFPPPPFPIPPFPPFPGPIIRGRR	JEMW01019668.1

		ABRR02179311.1
<i>Vicugna vicugna</i>	RRFFPRPFPFPGFPPFPFPIPIPIPFPGPIIRGRR	PNXW01021834.1
<i>Lama guanicoe</i>	RRFFPGFPPPGFPPFPFPIPIPIPFPGPIIRGRR	PNXV01010688.1
<i>Lama glama</i>	RRFFPGFPPPGFPPFPFPIPIPIPFPGPIIRGRR	PNXU01023268.1
Bac7-like cathelicidins (VicBac, CamBac, LamBac)		
<i>Camelus dromedarius</i>	RSIRRPRLPRPRVPRPYIPPRIPRPVLPFPPRFPIPRFPRGR	JDVD01078062.1
<i>Camelus ferus</i>	RSIRRPRLPRPRVPRPYIPPRIPRPVLPFPPRFPIPRFPRGR	AGVR01053366.1
<i>Camelus bactrianus</i>	RSIRRPRLPRPRVPRPYIPPRIPRPVLPFPPRFPIPRFPRGR	JARL01035149.1
<i>Vicugna pacos</i>	RRIRRPRLPRPRVPRPRIPPRIPRPVLPFPPRVFPFPRFPRGR	JEMW01005218.1 ABRR02002833.1
<i>Vicugna vicugna</i>	RRIRRPRLPRPRVPRPRIPPRIPRPVLPFPPRVFPFPRFPRGR	PNXW01021835.1
<i>Lama guanicoe</i>	RRIRRPRLPRPRVPRPHIPPRIPRPVLPFPPRVFPFRLRRR	PNXV01010685.1
<i>Lama glama</i>	RRIRRPRLPRPRVPRPHIPPRIPRPVLPFPPRVFPFRLRRR	PNXU01024686.1
Prophenin-like cathelicidins		
<i>Camelus dromedarius</i>	RFPVFNFPFPPNFPPQFPPNFPPRFPPNFPPNFPPPE FPLNFPPNFPPPEFPLNFPPPI	JDVD01076911.1
<i>Camelus dromedarius</i>	RFPAPNFPPNFPPPEFPLNFPPNFPPPEFPLNFPLNF FPPNFPPQFPPNFPPRFPPNFPPNFPPNFPPPEFPLNF PPNFPPPEFPLNFPPPI	JWIN01034417.1 LSZX01137301.1
<i>Camelus ferus</i>	RFPAPNFPPNFPPRFPPNFPPNFPPNFPPPEFPLNF FPPNFPPPEFPLNFPPPI	AGVR01059010.1
<i>Camelus bactrianus</i>	RFPAPNFPPNFPPRFPPNFPPNFPPNFPPPEFPLNF FPLNFPPNFPPRFPPNFPPNFPPNFPPPEFPLNF PPNFPPPEFPLNFPPPI	JARL01005656.1
<i>Vicugna pacos</i>	SFPPNFPPNFPPPEFPPNFPPNFPPRFPPNFPPNF PPPEFPLQNFPPPI	ABRR02179514.1
<i>Vicugna vicugna</i>	SFPPNFPPNFPPPEFPLQNFPPPI	PNXW01021834.1
<i>Vicugna vicugna</i>	SFPPNFPRFPFPPNFPPRFPPNFPPRFPPNFPPRF PPDFPPRFPPDFPPRFPPNFPPRFPPNFPPNFPP PEFPPNFPPNFPPNFPPPEFPLQNFPPPI	PNXW01017671.1
<i>Lama guanicoe</i>	SFPPNFPRFPFPPNFPRFPFPPNFPPRFPPNFPPRF PPNFPPNFPPNFPPRFPPNFPPPEFPPNFPPNF PPNFPPPEFPLQNFPPPI	PNXV01010685.1
Vp35-like cathelicidins (MAP-like)		
<i>Camelus dromedarius</i>	GLFGRIRDFHHDGVNWVRDKVGKVIYIGDKIRPG	JWIN01007525.1
<i>Camelus dromedarius</i>	GLFGRIRDSIRNRVNRVRDAIGKVIYVGDKIRPG	
<i>Camelus ferus</i>	GLFGRIRDFHHDGVNWVRDKVGKVIYIGDKIRPG	AGVR01065784.1
<i>Camelus bactrianus</i>	GLFGRIRDFHHDGVNWVRDKVGKVIYIGDKIRPG	JARL01035150.1
<i>Camelus bactrianus</i>	GLFGRIRDSIRKRVNRVRDAIGKVIYVGDKIRPG	JARL01005655.1
<i>Vicugna pacos</i>	GFFGRIHDFFRDGVNWVRDKVGKVIYIGDKIRPG	JEMW01026878.1 ABRR02002839.1
<i>Vicugna vicugna</i>	GFFGRIHDFFRDGVNWVRDKVGKVIYIGDKIRPG	PNXW01021834.1
	GVFGRIRNFFRDRVNRVRDTVGVKVIYIGDKIRPG	PNXW01021834.1
<i>Lama guanicoe</i>	GFFGGIRDFFRDGVNRVRDTVGVKVIYIGDKIRPG	PNXV01022928.1
	RLFGGIRDFIRDRVNRVRDKVGKVIYIGDKIRPG	PNXV01010688.1
<i>Lama glama</i>	GFFGGIRDFFRDGVNRVRDTVGVKVIYIGDKIRPG	PNXU01023268.1