Supporting Information

High-throughput screening for substrate specificity-adapted mutants of the nisin dehydratase NisB

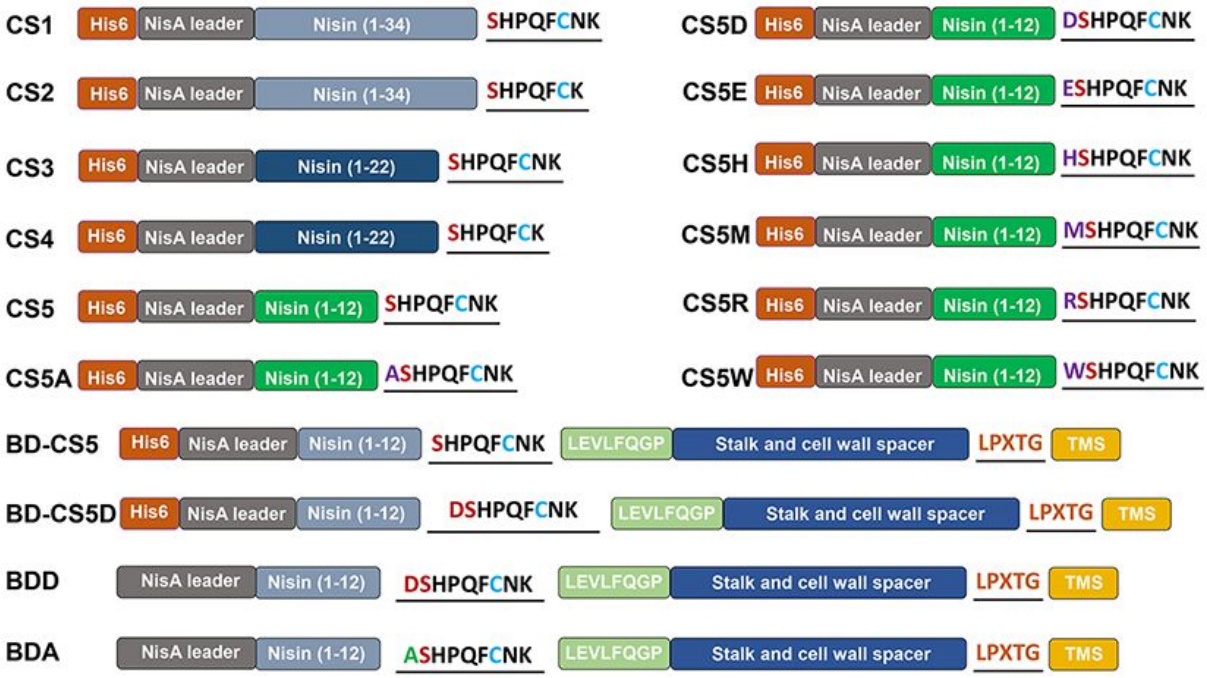
Xinghong Zhao [1, 2], Rubén Cebrián [1], Yuxin Fu [1], Rick Rink [3], Tjibbe Bosma [3], Gert N. Moll [1,3] and Oscar P. Kuipers [1,*]

[1] Department of Molecular Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen 9747 AG, The Netherlands.

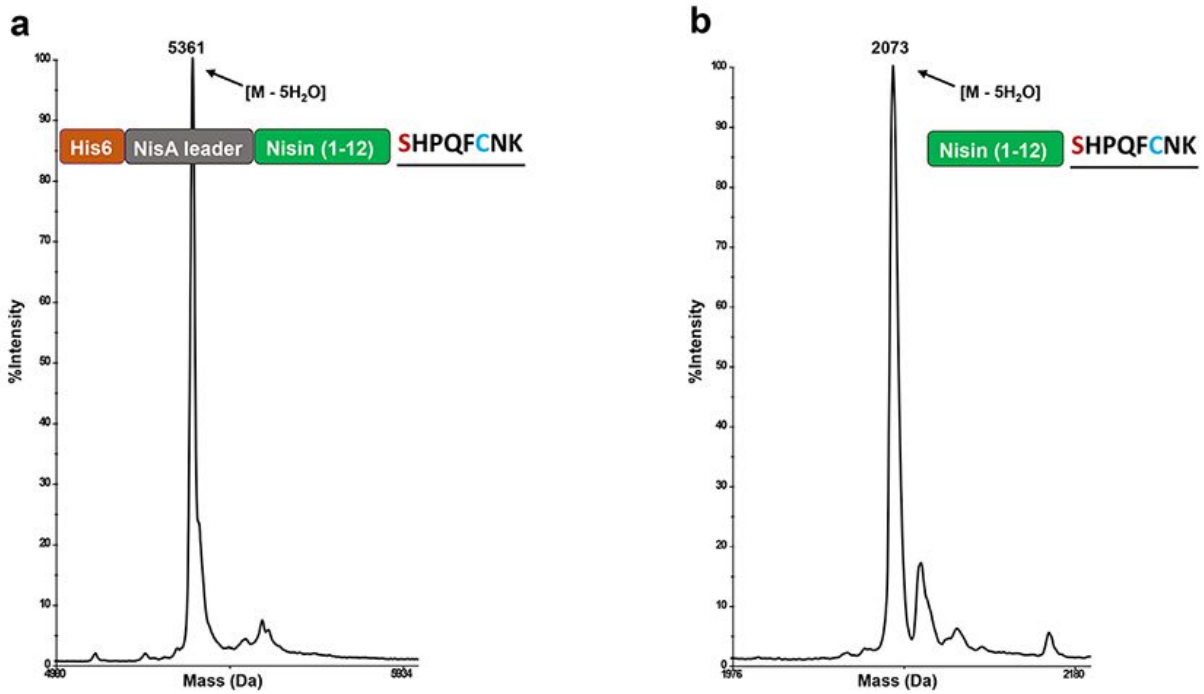
[2] Natural Medicine Research Center, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China.

[3] Lanthio Pharma, Rozenburglaan 13 B, Groningen 9727 DL, The Netherlands.

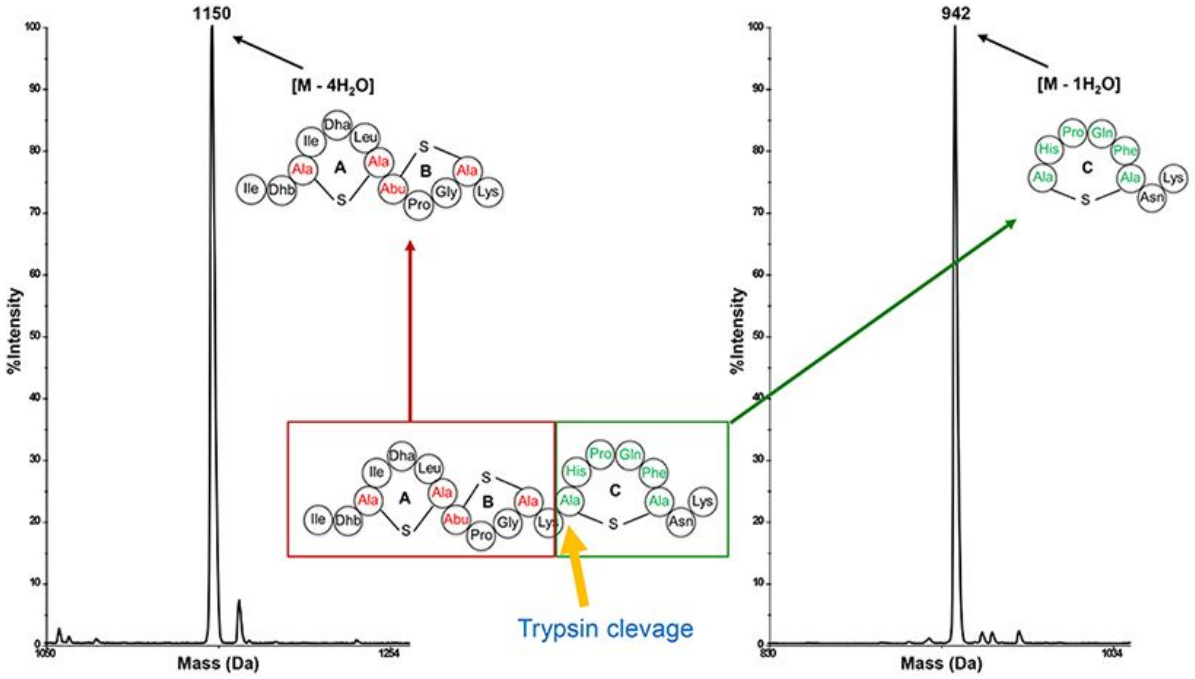
* Correspondence: o.p.kuipers@rug.nl (Oscar P. Kuipers)



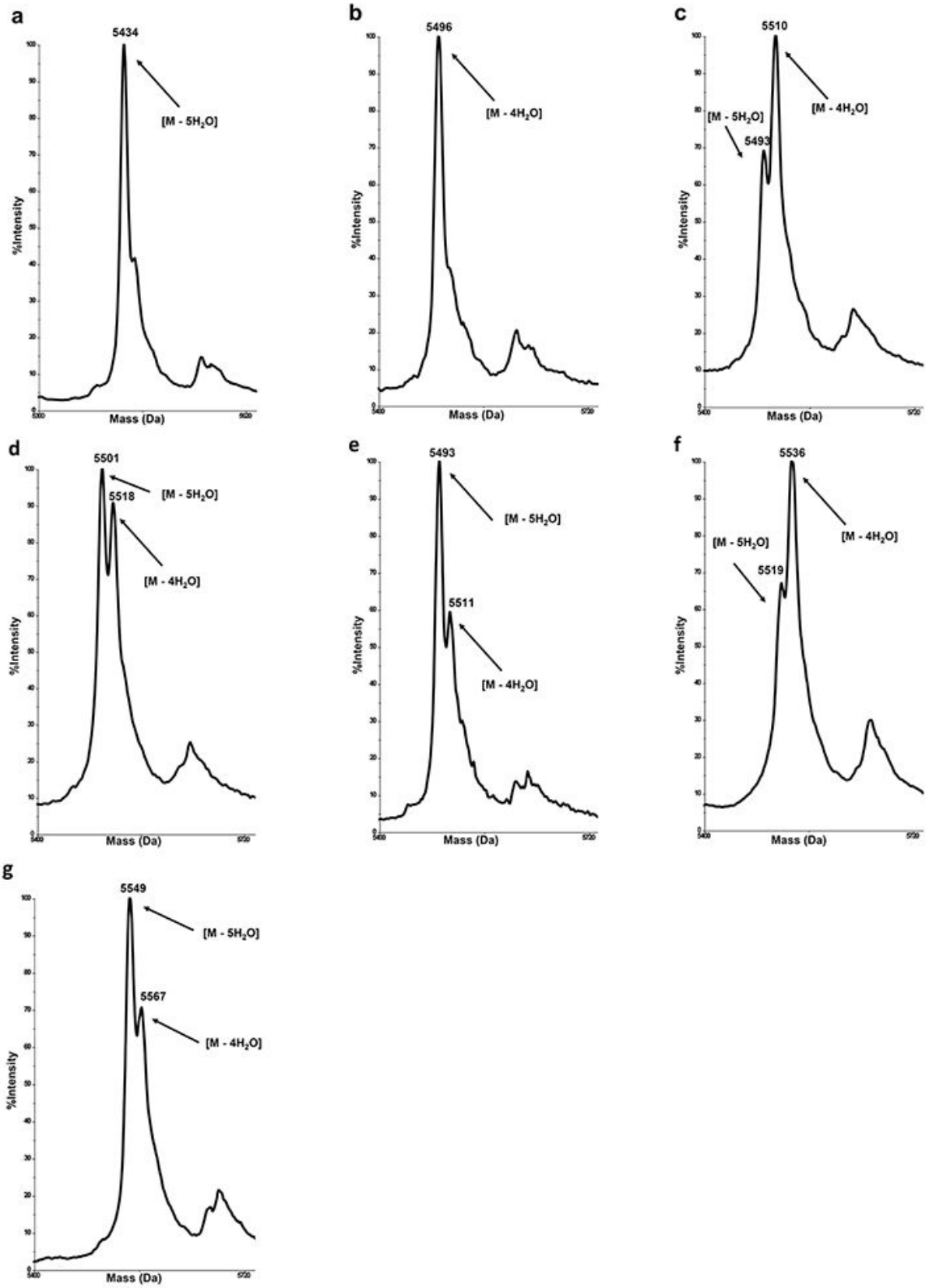
Supplemental Figure 1. Amino acid sequence of designed peptides.



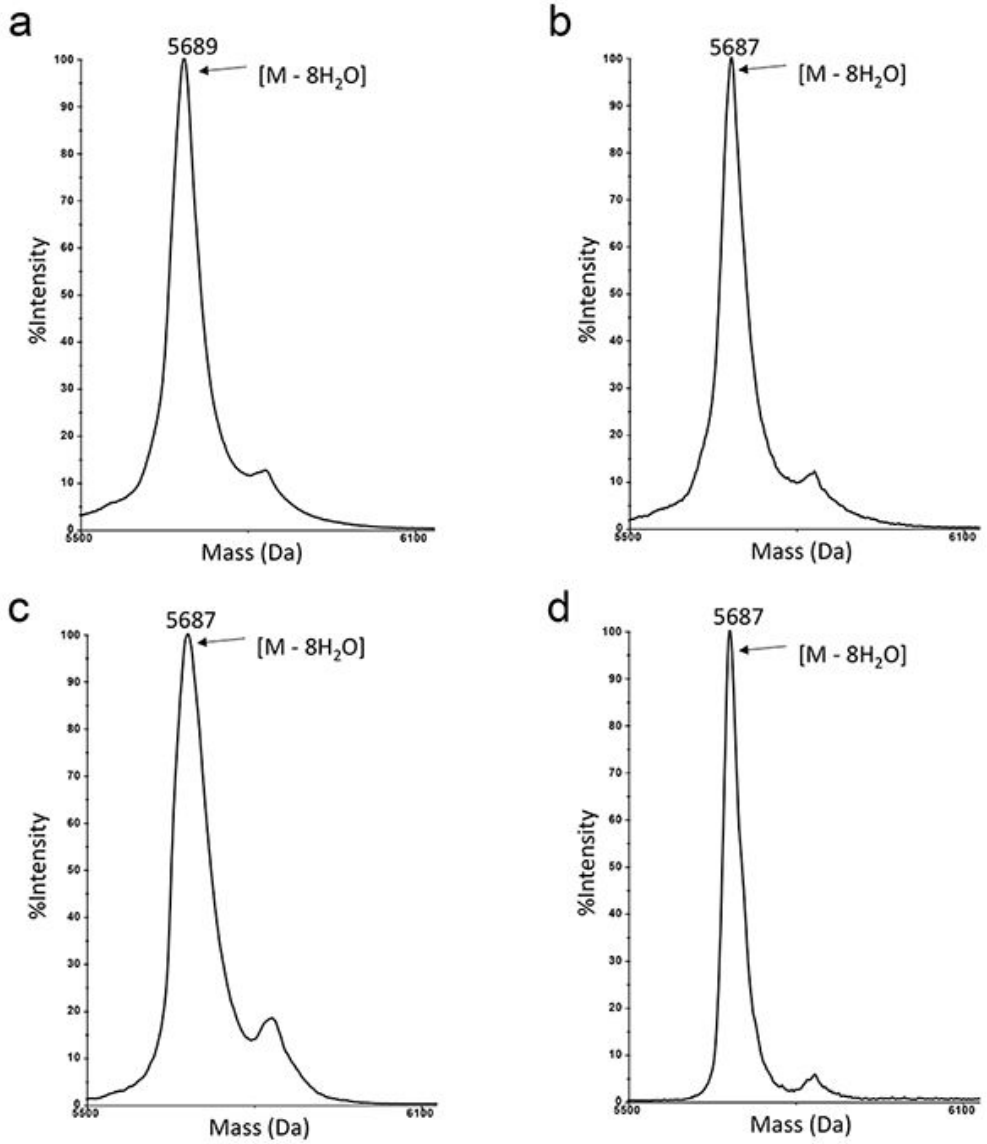
Supplemental Figure 2. MALDI-TOF MS data of CS5 before (**a**) and after (**b**) cleavage of the leader peptide.



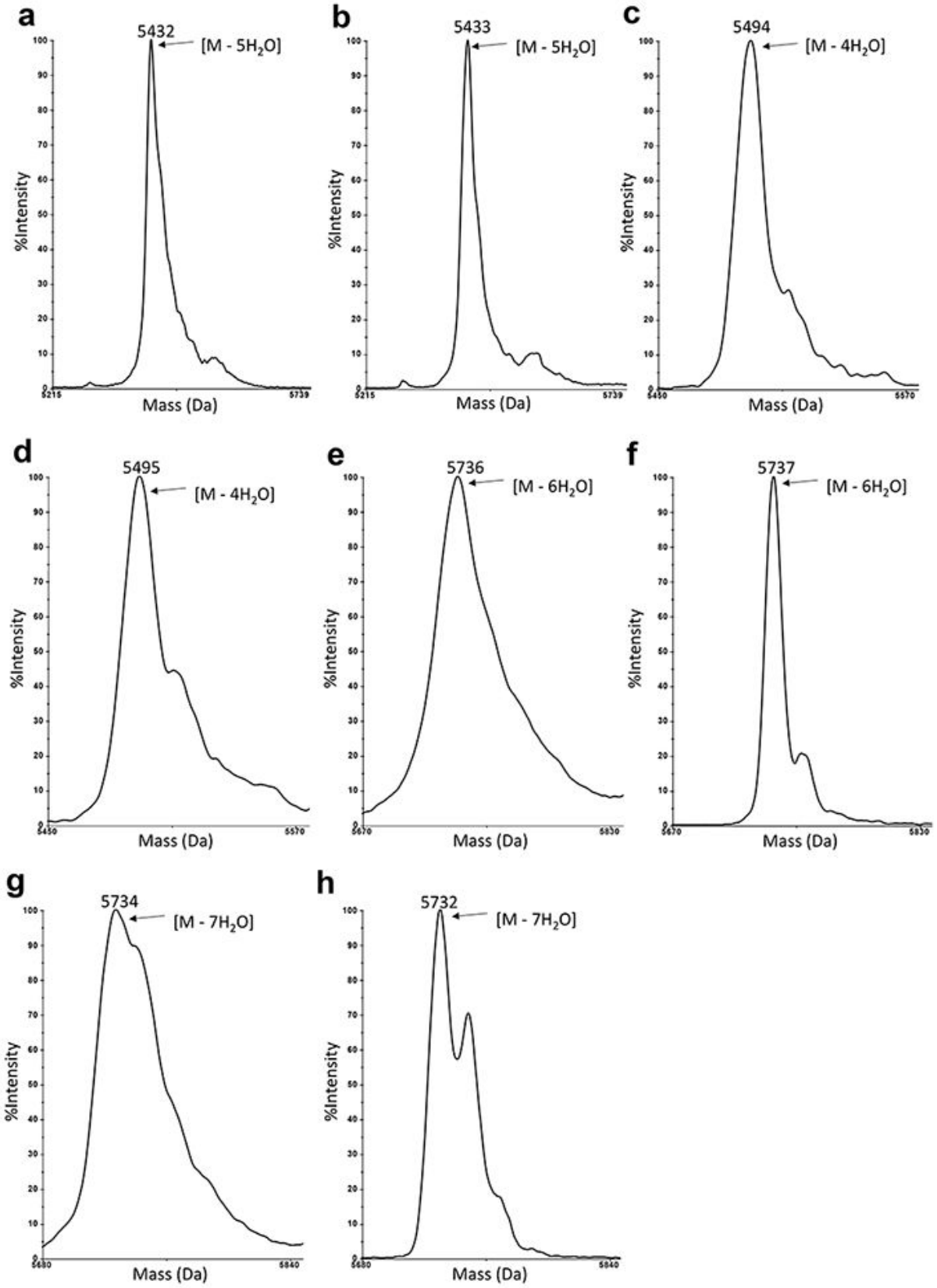
Supplemental Figure 3. MALDI-TOF MS data of trypsin treated CS5 fragments. It demonstrates that the cyclic strep-ligand was correctly formed since CS5 was cleaved by trypsin after Lys12, liberating the cyclic strep-ligand.



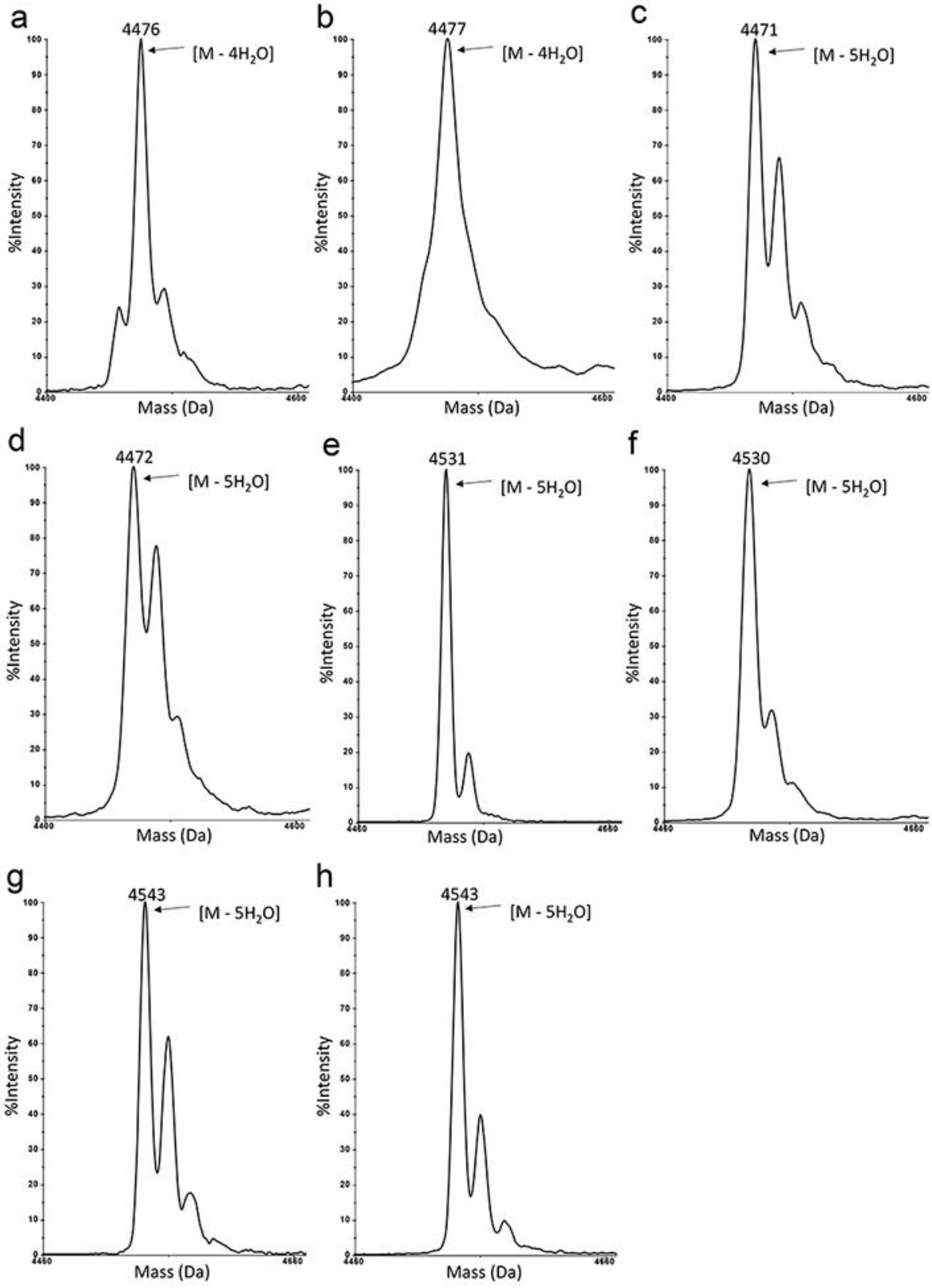
Supplemental Figure 4. MALDI-TOF MS data of CS5A (**a**), CS5D (**b**), CS5E (**c**), CS5H (**d**), CS5M (**e**), CS5R (**f**), CS5W (**g**).



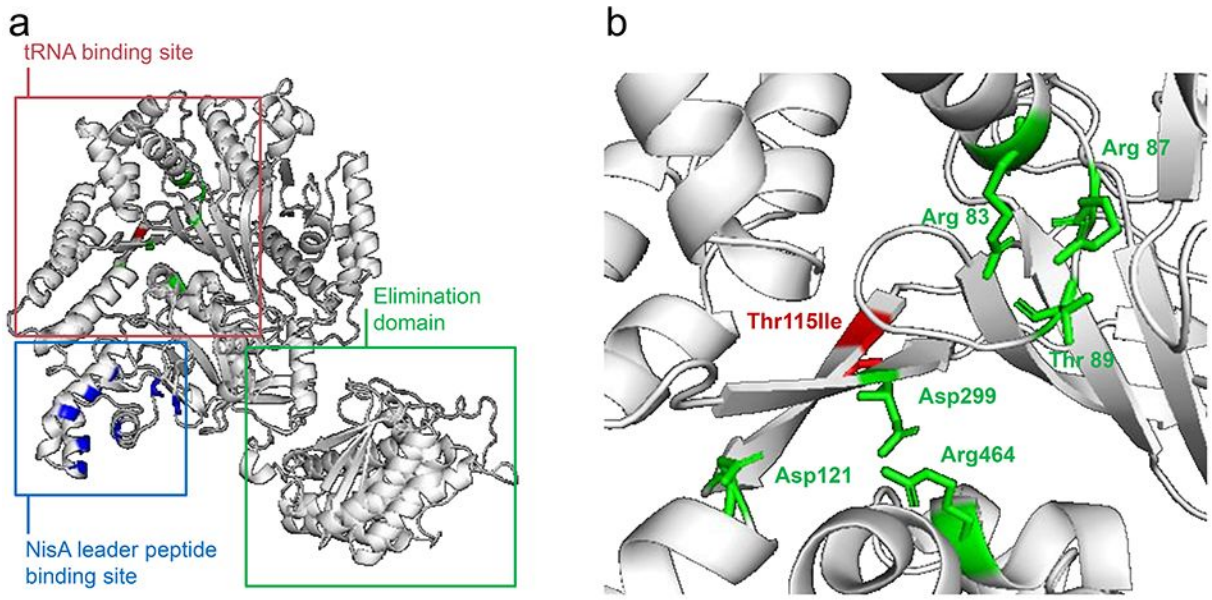
Supplemental Figure 5. MALDI-TOF MS data of nisin modified by NisB mutants. **a**, modified by $NisB^{wt}$; **b**, modified by $NisB^{S88P/D234N}$; **c**, modified by $NisB^{S88P}$; **d**, modified by $NisB^{D234N}$.



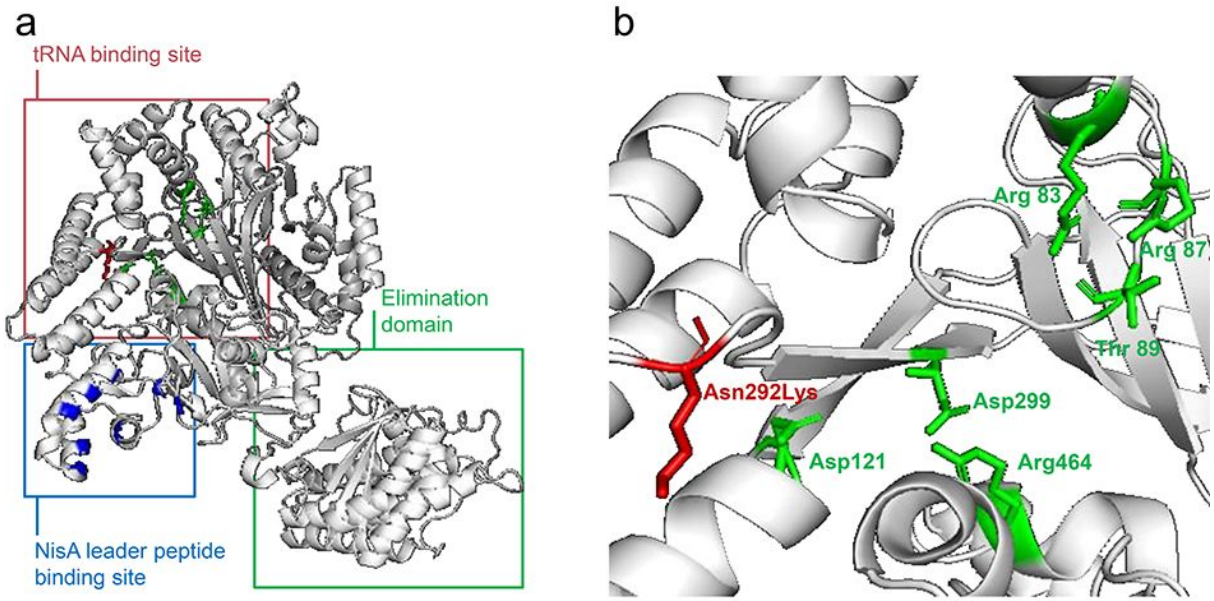
Supplemental Figure 6. MALDI-TOF MS data of peptides modified by NisB mutants. **a**, CS5A, modified by $NisB^{S88P}$; **b**, CS5A, modified by $NisB^{D234N}$; **c**, CS5D, modified by $NisB^{S88P}$; **d**, CS5D, modified by $NisB^{D234N}$; **e**, $nisin^{T2D}$, modified by $NisB^{S88P}$; **f**, $nisin^{T2D}$, modified by $NisB^{D234N}$; **g**, $nisin^{T2E}$, modified by $NisB^{S88P}$; **h**, $nisin^{T2E}$, modified by $NisB^{D234N}$.



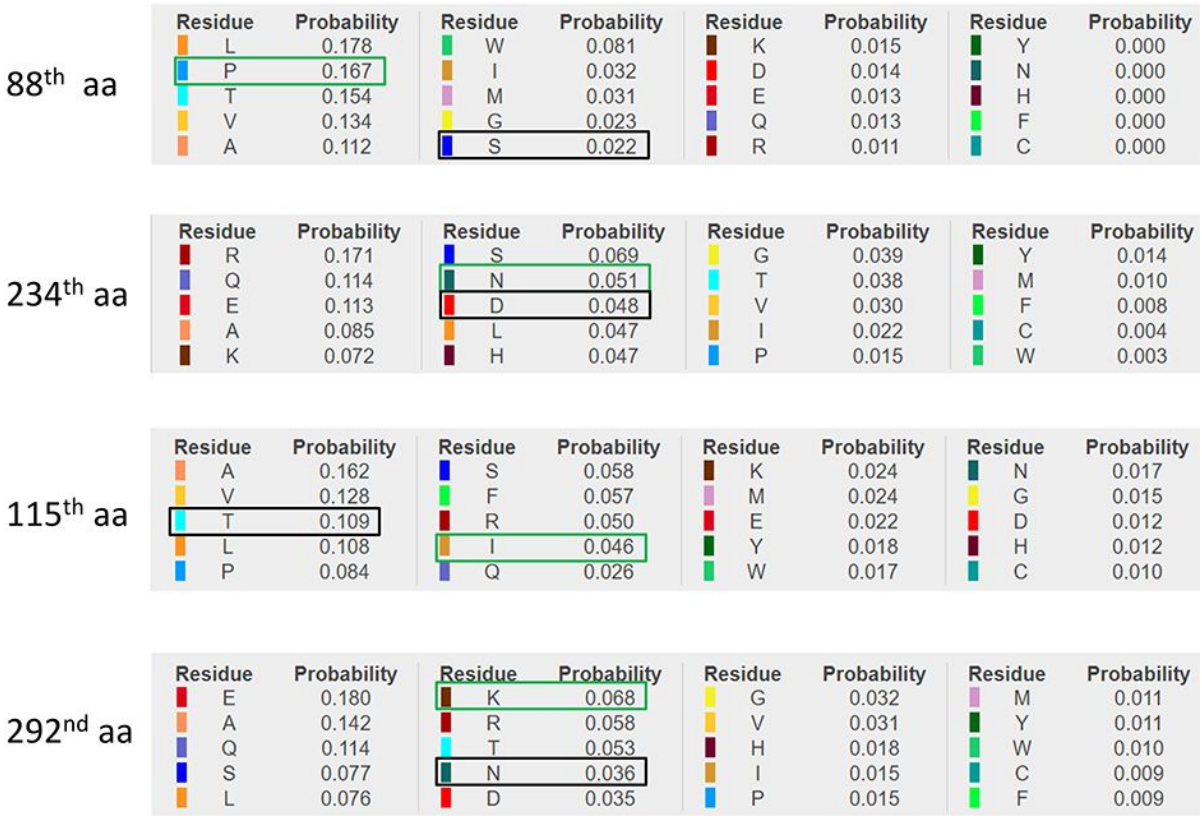
Supplemental Figure 7. MALDI-TOF MS data of peptides modified by NisB mutants. **a**, nisin(1-22)^{K12D}, modified by NisB^{S88P}; **b**, nisin(1-22)^{K12D}, modified by NisB^{D234N}; **c**, nisin(1-22)^{K12E}, modified by NisB^{S88P}; **d**, nisin(1-22)^{K12E}, modified by NisB^{D234N}; **e**, nisin(1-22)^{G14D}, modified by NisB^{S88P}; **f**, nisin(1-22)^{G14D}, modified by NisB^{D234N}; **g**, nisin(1-22)^{G14E}, modified by NisB^{S88P}; **h**, nisin(1-22)^{G14E}, modified by NisB^{D234N}.



Supplemental Figure 8. a, Overall structure of the NisB dehydratase and the functions of each domain [1]. **b**, glutamyl-tRNA^{Glu} binding site. Amino acids are shown in green that are known to be important for tRNA binding; mutation points of NisB^{T115I} are show in red.



Supplemental Figure 9. a, Overall structure of the NisB dehydratase and the functions of each domain [1]. **b**, glutamyl-tRNA^{Glu} binding site. Amino acids are shown in green that are known to be important for tRNA binding; mutation points of NisB^{N292K} are show in red.



Supplemental Figure 10. LanB-alignment-based probability of amino acid occurrence in positions 88, 234, 115 and 292. Following the obtention of S88P, D234N, T115I, N292K mutations in NisB, the LanB family were aligned based on the full model of the N terminal domains (PFAM PF04737 http://pfam.xfam.org/family/Lant_dehyd_N). The figure depicts the probability of the occurrence of each amino acid in the four positions. Clearly none of the four positions is conserved. Wild type amino acid is marked by black rectangle; amino acid of mutation is marked by a green rectangle. In positions 88, 234 and 292 the probability of the new amino acid is higher than of the wild type amino acid.

Supplemental Table 1. Stains and plasmids used in this study.

Strain or plasmid	Characteristics	Source or reference
Strains		
L. lactis NZ9000	MG1363 derivative; NisRK+	2
E. coli XL-10 gold	XL10-Gold Ultracompetent Cells	Agilent Technologies
Plasmids		
pNZnisA leader his2	pNZ8048 derivative, nisA, Cm^r, encoding his6 residues behind the first Met gene of nisin leader	3
pCS1	pNZ8048 derivative, CS1, Cm^r	This work
pCS2	pNZ8048 derivative, CS2, Cm^r	This work
pCS3	pNZ8048 derivative, CS3, Cm^r	This work
pCS4	pNZ8048 derivative, CS4, Cm^r	This work
pCS5	pNZ8048 derivative, CS5, Cm^r	This work
pCS5A	pNZ8048 derivative, CS5A, Cm^r	This work
pCS5D	pNZ8048 derivative, CS5D, Cm^r	This work
pCS5E	pNZ8048 derivative, CS5E, Cm^r	This work
pCS5H	pNZ8048 derivative, CS5H, Cm^r	This work
pCS5M	pNZ8048 derivative, CS5M, Cm^r	This work
pCS5R	pNZ8048 derivative, CS5R, Cm^r	This work
pCS5W	pNZ8048 derivative, CS5W, Cm^r	This work
pBD-CS5	pNZ8048 derivative, BD-CS5, Cm^r	This work
pBDA	pNZ8048 derivative, BDA, Cm^r	This work
pBD-CS5D	pNZ8048 derivative, BD-CS5D, Cm^r	This work
pBDD	pNZ8048 derivative, BDD, Cm^r	This work
pNZnisA	pNZ8048 derivative, Nisin, Cm^r	4
$pNisin^{T2D}$	pNZ8048 derivative, $Nisin^{T2D}$, Cm^r	5
$pNisin^{T2E}$	pNZ8048 derivative, $Nisin^{T2E}$, Cm^r	5
pNisin(1-22)	pNZ8048 derivative, Nisin(1-22), Cm^r	5
$pNisin(1-22)^{K12D}$	pNZ8048 derivative, $Nisin(1-22)^{K12D}$, Cm^r	This work
$pNisin(1-22)^{K12E}$	pNZ8048 derivative, $Nisin(1-22)^{K12E}$, Cm^r	This work
$pNisin(1-22)^{G14D}$	pNZ8048 derivative, $Nisin(1-22)^{G14D}$, Cm^r	This work
$pNisin(1-22)^{G14E}$	pNZ8048 derivative, $Nisin(1-22)^{G14E}$, Cm^r	This work
pTLR-BTC	P_{nis} nisBTC, Em^r	6

Supplemental Table 2. Primers for PCRs used in this study.

Plasmids	Templates	Primers	Nucleic acid sequences (5' to 3')	Characteristics (5'-lable)
pCS1	pNZnisA	PXZ1	AAGCTTTCTTTGAACCAAAATTAGAAAACCAAG	5'- phosphorylation
		PXZ2	ATTTGTTACAAAATTGTGGGTGTGATTTGCTTAC GTGAATACTACAATGACAAGTTG	
pCS2	pNZnisA	PXZ1	AAGCTTTCTTTGAACCAAAATTAGAAAACCAAG	5'- phosphorylation
		PXZ3	ATTTACAAAATTGTGGGTGTGATTTGCTTACGTG AATACTACAATGACAAGTTG	
pCS3	pNZnisA	PXZ1	AAGCTTTCTTTGAACCAAAATTAGAAAACCAAG	5'- phosphorylation
		PXZ4	ATTTGTTACAAAATTGTGGGTGTGATTTCATGTT ACAACCCATCAGAGCTC	
pCS4	pNZnisA	PXZ1	AAGCTTTCTTTGAACCAAAATTAGAAAACCAAG	5'- phosphorylation
		PXZ5	ATTTACAAAATTGTGGGTGTGATTTCATGTTACA ACCCATCAGAGCTC	
pCS5	pNZnisA	PXZ1	AAGCTTTCTTTGAACCAAAATTAGAAAACCAAG	5'- phosphorylation
		PXZ6	ATTTGTTACAAAATTGTGGGTGTGATTTACAACC GGGTGTACATAGCGAAATAC	
pCS5A/ pBDA	pCS5/ pBDD	PXZ7	TTTACAACCGGGTGTACATAGCGAAATAC	5'- phosphorylation
		PXZ8	GCTTCACACCCACAATTTTGTAACAAATAAGCTTTC	
pCS5D/ pBD-CS5D	pCS5/ pBD-CS5	PXZ7	TTTACAACCGGGTGTACATAGCGAAATAC	5'- phosphorylation
		PXZ9	GATTCACACCCACAATTTTGTAACAAATAAGCTTTC	
pCS5E	pCS5	PXZ7	TTTACAACCGGGTGTACATAGCGAAATAC	5'- phosphorylation
		PXZ10	GAATCACACCCACAATTTTGTAACAAATAAGCTTTC	
pCS5H	pCS5	PXZ7	TTTACAACCGGGTGTACATAGCGAAATAC	5'- phosphorylation
		PXZ11	CACTCACACCCACAATTTTGTAACAAATAAGCTTTC	
pCS5M	pCS5	PXZ7	TTTACAACCGGGTGTACATAGCGAAATAC	5'- phosphorylation
		PXZ12	ATGTCACACCCACAATTTTGTAACAAATAAGCTTTC	
pCS5R	pCS5	PXZ7	TTTACAACCGGGTGTACATAGCGAAATAC	5'- phosphorylation
		PXZ13	AGATCACACCCACAATTTTGTAACAAATAAGCTTTC	
pCS5W	pCS5	PXZ7	TTTACAACCGGGTGTACATAGCGAAATAC	5'- phosphorylation
		PXZ14	TGGTCACACCCACAATTTTGTAACAAATAAGCTTTC	
pBDD	pBD-CS5D	PXZ15	GTGATGATGCATGGTGAGTGCCTCCTTATAATTTATTTTG	5'- phosphorylation
		PXZ16	CATCACCATAGTACAAAAGATTTTAACTTGGATTTGGTAT CTG	
pNisin(1-22)^{K12D}	pNisin(1-22)	PXZ17	ACAACCGGGTGTACATAGCGAAATAC	5'- phosphorylation
		PXZ18	GATACAGGAGCTCTGATGGGTTGTAAC	
pNisin(1-22)^{K12E}	pNisin(1-22)	PXZ17	ACAACCGGGTGTACATAGCGAAATAC	5'- phosphorylation
		PXZ19	GAAACAGGAGCTCTGATGGGTTGTAAC	
pNisin(1-22)^{G14D}	pNisin(1-22)	PXZ20	ATTTGTTACAAAATTGTGGGTGTGATTTACAACCGG GTGTACATAGCGAAATAC	5'- phosphorylation
		PXZ21	ACAGATGCTCTGATGGGTTGTAACATGAAATAAG	
pNisin(1-22)^{G14E}	pNisin(1-22)	PXZ20	ATTTGTTACAAAATTGTGGGTGTGATTTACAACCGG GTGTACATAGCGAAATAC	5'- phosphorylation
		PXZ22	ACAGAAGCTCTGATGGGTTGTAACATGAAATAAG	

Supplemental Table 3. Primers for PCRs used in this study.

Primers	Nucleic acid sequences (5' to 3')	Purpose
Pbd1	GAAGTTCTGTTTCAAGGACCCTTGCAGGCAGCTAAGCAGGAAC	To construct the plasmid that encoding the gene of bacterial display protein BD-CS5
Pbd2	CTAATTTTGGTTCAAAGAAAGCTTATTCTTCACGTTGTTTCCGTTTCAATC	
Pbd3	CAGCTCCAAGATCTAGTCTTATAACTATACTGAC	
Pbd4	CAAGGGTCCTTGAAACAGAACTTCCAATTTGTTACAAAATTGTGGGTGTGATTTAC	
Pbd5	GAAGAATAAGCTTTCTTTGAACCAAAATTAGAAAACCAAG	
Pbd6	CTTAGCAATAGCGTCCTTTGATTCATG	
Ptlr1	AACTTTTTATCAUGTTTTTTCCTCTCTTTATTTTTATAAGC	NisB library construction
Ptlr2	ATACATGAAAUGAGGACTAATAGATGGATGAAGTGAAAG	
Ptlr3	ATGATAAAAAGTUCATTTAAAGCTCAACCGTTTTTAG	
Ptlr4	ATTTCATGTAUTCTTCCGAAACAAACAACCTTTGAAGTGTG	
PS1	CTATCAATCAAAGCAACACGTGC	Sequencing
PS2	GATAACGCGAGCATAATAAACGG	
PS3	CTCCAAACGATAAACGGAGTTTTAC	
PS4	AACCCTTTTCTTCTTTAACAGACCAGC	
PS5	GATTTGTTGTCAGATTTTTCTTGGAAC	
PS6	GAATTTGGCAAAGGAGTATGAAAAAG	
PS7	CCCAAAGAGTTTTATATTGTCAATGG	
PS8	CGAAGCAATATTTTGTGCCGATTC	

References

(1) Ortega, M. A.; Hao, Y.; Zhang, Q.; Walker, M. C.; van der Donk, W. A.; Nair, S. K. Structure and Mechanism of the TRNA-Dependent Lantibiotic Dehydratase NisB. Nature 2015, 517 (7535), 509.

(2) Kuipers, O. P.; de Ruyter, P. G. G. A.; Kleerebezem, M.; de Vos, W. M. Controlled Overproduction of Proteins by Lactic Acid Bacteria. *Trends Biotechnol*. **1997**, 15 (4), 135–140.

(3) Li, Q.; Montalban-Lopez, M.; Kuipers, O. P. Increasing the Antimicrobial Activity of Nisin-Based Lantibiotics against Gram-Negative Pathogens. *Appl. Environ. Microbiol.* **2018**, 84 (12), e00052-18.

(4) Zhou, L.; van Heel, A. J.; Montalban-Lopez, M.; Kuipers, O. P. Potentiating the Activity of Nisin against Escherichia Coli. *Front. cell Dev. Biol.* **2016**, 4, 7.

(5) Zhao, X.; Yin, Z.; Breukink, E.; Moll G.N.; Kuipers, O. P. An engineered double lipid II binding motifs-containing lantibiotic displays potent and selective antimicrobial activity against *E. faecium*. *Antimicrob. Agents Chemother*. (**2020**).

(6) Cebrian, R.; Macia-Valero, A.; Jati, A. P.; Kuipers, O. P. Design and Expression of Specific Hybrid Lantibiotics Active against Pathogenic Clostridium Spp. *Front. Microbiol*. **2019**, 10.