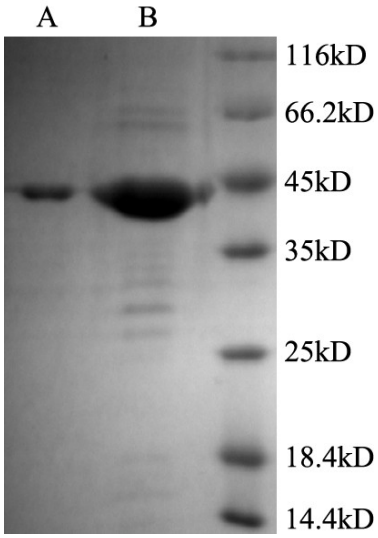
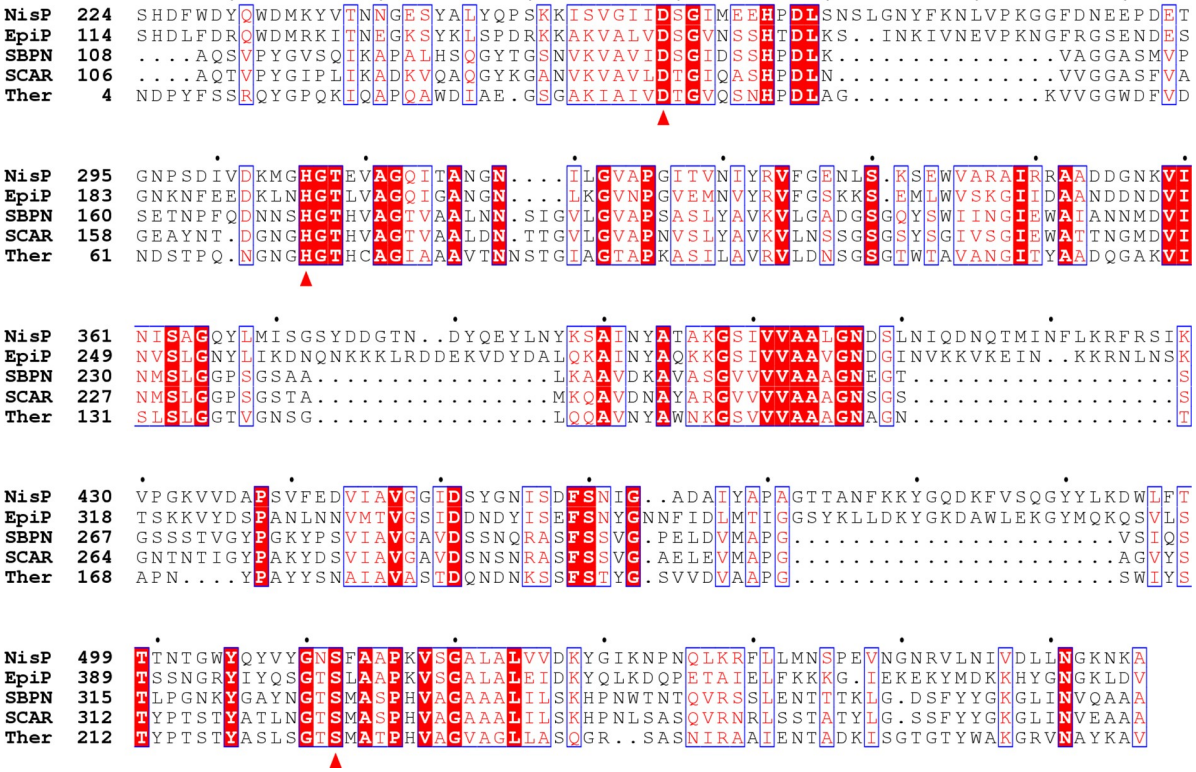
Supplementary Material

Supplementary Figure S1. SDS-PAGE gel of the crystals of NisP (lane B) and the catalytic domain of NisP (residues 224-566) with active site mutant S512A (lane A). The mass similarity between lane A and B indicates the missing residues 196-223, 567-634 and 648-682 in our final model result from the auto-cleavage and degradation in crystallisation process.

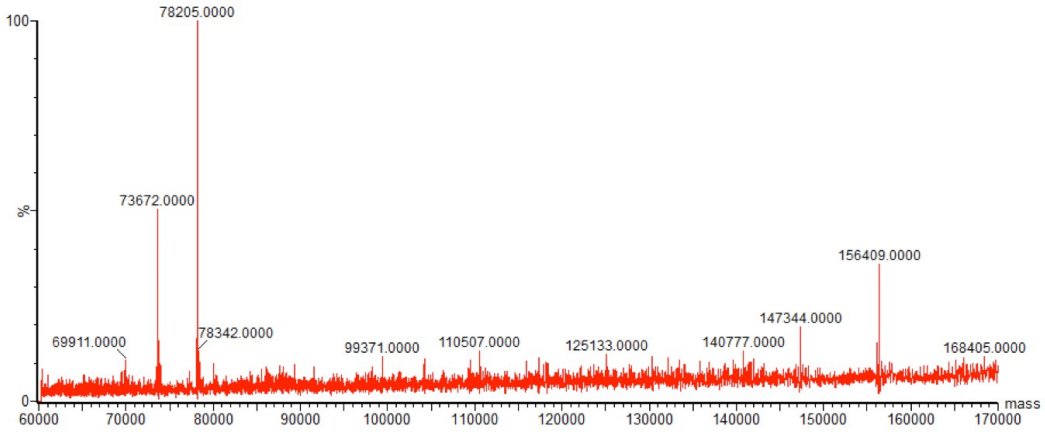


Supplementary Figure S2. Multiple sequence alignment of NisP and the homologous subtilases. From top to bottom, the sequences are NisP, epidermin leader peptidase from *S. epidermidis* (marked as EpiP), subtilisin BPN' from *Bacillus amyloliquefaciens* (SBPN), subtilisin Carlsberg from *Bacillus licheniformis* (SCAR) and thermitase from *Thermoactinomyces vulgaris* (Ther). Identical residues are highlighted with a red background, and conserved residues are coloured red. The conserved active site residues are labelled with red triangles. The conserved region was defined by NCBI BLAST. The sequences were aligned using the program ClustalW, and the alignment was represented using the online ESPript 2.0 server.

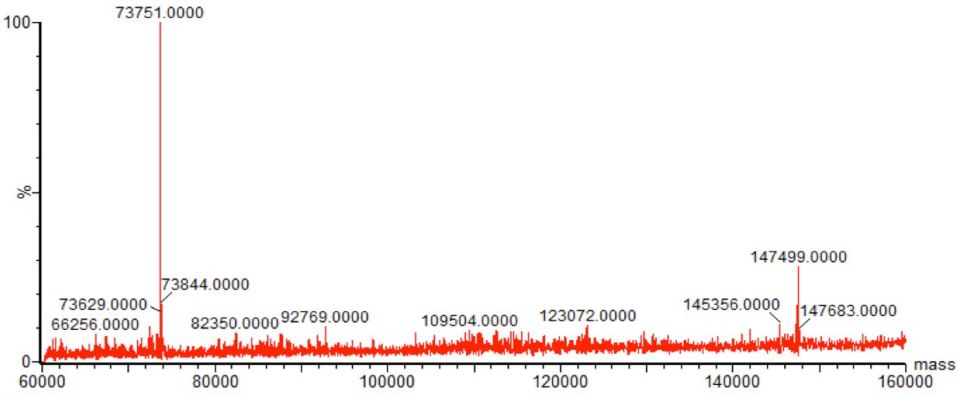


Supplementary Figure S3. Native protein mass spectrometry analysis of purified double-tagged wild-type mature NisP (B) and active site mutant H306A (A). The molecular weight differences calculated by MS (78205 kD-73751 kD=4454 kD) verified the cleavage after Arg647. Notably, the peak showing the molecular weight of 73672 kD in panel A indicates the partial enzymatic activity retained in this mutant.

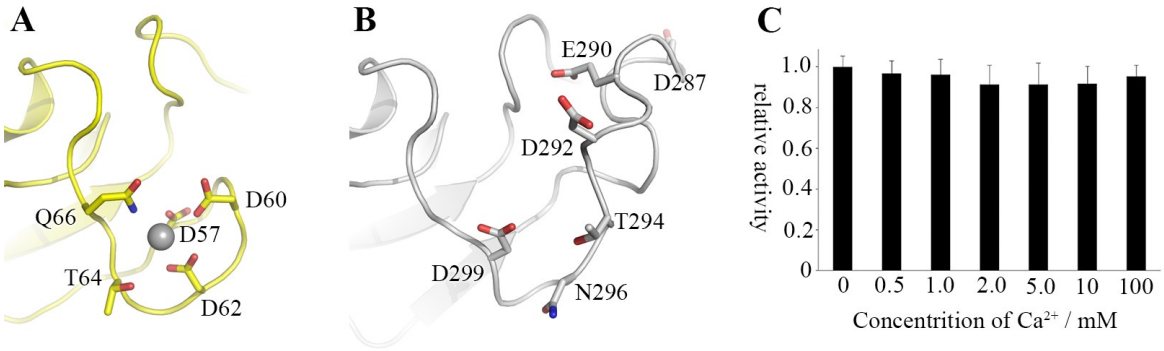
A



B



Supplementary Figure S4. Comparison of the calcium binding site of thermitase from *Thermoactinomyces vulgaris* (A) and the predicted calcium binding site of mature NisP (B). In the structure of thermitase, the related residues are distributed surrounding the metal ion and forming a good calcium binding coordination system. However, the predicted calcium chelating residues in NisP exhibit a linear configuration that cannot form a metal ion coordination system. The crystal structures of thermitase and NisP are coloured yellow and white, respectively. (C) Effect of calcium on the activity of NisP. After purification, the protein was treated with 0.1 M EDTA, desalted by superdex75 column, and then used to perform the activity assay. There's no significant effect on the enzyme activity after stepwise addition of calcium (up to 0.1 M) into the EDTA-treated NisP.



Supplementary Table S1. Growth condition comparison of wild type and RS2P mutant.

Culture time/h	$OD_{Wildtype}$[a]	OD_{RS2P}[a]	ΔOD[b]	ΔOD%[c]
0	0.08±0.002	0.10±0.001	-0.01	-15.8
5	0.25±0.004	0.22±0.009	0.03	10.9
6	0.53±0.011	0.46±0.006	0.07	13.1
7	0.80±0.005	0.72±0.013	0.08	10.0
8	1.45±0.011	1.17±0.012	0.28	19.2
9	1.86±0.024	1.57±0.015	0.29	15.4
10	2.09±0.049	1.89±0.023	0.20	9.6
11	2.49±0.014	2.32±0.018	0.17	6.8
12	2.53±0.005	2.42±0.006	0.12	4.7

[a] OD values were measured at 600 nm for at least three times and mean values are shown in the table.
[b] $\Delta OD = OD_{Wild\ type} - OD_{RS2P}$
[c] $\Delta OD\% = \Delta OD / OD_{Wild\ type}$