

Supplemental Table S1. **Plasmids used in this study**

Plasmid	Genotype	Source
pUC-119	<i>bla</i> $\Delta$ <i>lacZ</i>	Takara
pQE-30	<i>bla</i>	Qiagen
pUC-SLT	<i>bla</i> $\Delta$ <i>lacZ</i> $\Delta$ <i>cwlP</i> (SLT domain of CwlP)	This study
pQE-SLT	<i>bla</i> (His) <sub>6</sub> - $\Delta$ <i>cwlP</i> (SLT domain of CwlP)	This study
pQE-M23	<i>bla</i> (His) <sub>6</sub> - $\Delta$ <i>cwlP</i> (peptidase M23 domain of CwlP)	This study
pQE-SLTM23	<i>bla</i> (His) <sub>6</sub> - $\Delta$ <i>cwlP</i> (SLT-peptidase M23 domains of CwlP)	This study
pQE-YomI-FL	<i>bla</i> (His) <sub>6</sub> - <i>cwlP</i> (entire region of CwlP)	This study
pQESLT-E1447Q	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-SLT, E1447Q)	This study
pQESLT-E1447A	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-SLT, E1447A)	This study
pQESLT-S1448A	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-SLT, S1448A)	This study
pQESLT-S1456A	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-SLT, S1456A)	This study
pQESLT-Q1465A	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-SLT, Q1465A)	This study
pQESLT-D1481N	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-SLT, D1481N)	This study
pQESLT-Q1484L	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-SLT, Q1484L)	This study
pQESLT-N1485A	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-SLT, N1485A)	This study
pQESLT-N1512A	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-SLT, N1512A)	This study
pQESLT-E1529Q	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-SLT, E1529Q)	This study
pQEM23-H1580Q	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-M23, H1580Q)	This study
pQEM23-D1584N	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-M23, D1584N)	This study
pQEM23-N1612A	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-M23, N1612A)	This study
pQEM23-H1628Q	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-M23, H1628Q)	This study
pQEM23-Q1645A	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-M23, Q1645A)	This study
pQEM23-S1656A	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-M23, S1656A)	This study
pQEM23-H1660Q	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-M23, H1660Q)	This study
pQEM23-H1662Q	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-M23, H1662Q)	This study
pQEM23-N1668A	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-M23, N1668A)	This study
pQEM23-D1673A	<i>bla</i> (His) <sub>6</sub> -mutated $\Delta$ <i>cwlP</i> (mutated CwlP-M23, D1673A)	This study

Supplemental Table S2. **Primers used in this study**

Primer name	Sequence (5'->3')	Construct name
BF-SLT	<i>gccgggatcc</i> GGCAAGTATTCAAGCTACA	pQE-SLT pQE-SLTM23
KR-SLT	<i>gcgcggtacc</i> GGCCATGATCTTCTTGAC	pQE-SLT
BF-M23	<i>gccgggatcc</i> AAGAAGATCATGGCCAAC	pQE-M23
HR-M23	<i>gcgcaagctt</i> TTTAGCCTGAGCTATCCC	pQE-M23
KR-M23	<i>gcgcggtacc</i> TTTAGCCTGAGCTATCCC	pQE-SLTM23
BF-YomI-FL	<i>gccgggatcc</i> TTGAGTCAAAACCTCAAAATT	pQE-YomI-FL
KR-YomI-FL	<i>cgcggtacc</i> CATGCTACCATTTAGTTTTAC	pQE-YomI-FL
E1447Q-1	GCAGCTGTAATTCAGCAAc <u>AA</u> TCAGGGTTTAATGC	pQESLT-E1447Q
E1447Q-2	GCATTAAACCCTGATTgTTGCTGAATTACAGCTGC	pQESLT-E1447Q
E1447A-1	GCAGCTGTAATTCAGCAAG <u>cA</u> TCAGGGTTTAATGC	pQESLT-E1447A
E1447A-2	GCATTAAACCCTGATgCTTGCTGAATTACAGCTGC	pQESLT-E1447A
S1448A-1	GCTGTAATTCAGCAAGAAg <u>CA</u> GGGTTTAATGCTAAAG	pQESLT-S1448A
S1448A-2	CTTTAGCATTAAACCCTGcTTCTTGCTGAATTACAGC	pQESLT-S1448A
S1456A-1	GCTAAAGCACGA <u>gC</u> TGGTGTAGGTGCC	pQESLT-S1456A
S1456A-2	GGCACCTACACCAGcTCGTGCTTTAGC	pQESLT-S1456A
Q1465A-1	CCATGGGATTAATG <u>gcA</u> CTGATGCCAGCAAC	pQESLT-Q1465A
Q1465A-2	GTTGCTGGCATCAGTgcCATTAAATCCCATGG	pQESLT-Q1465A
D1481N-1	GGAGTAAATAACGCTTAC <u>aA</u> TCTTATCAAAATGTTATGGGTG	pQESLT-D1481N
D1481N-2	CACCCATAACATTTTGATAAGGA <u>AT</u> tGTAAGCGTTATTTACTCC	pQESLT-D1481N
Q1484L-1	CGCTTACGATCCTTATC <u>tA</u> AAATGTTATGGGTGGAAC	pQESLT-Q1484L
Q1484L-2	GTTCCACCCATAACATTTaGATAAGGATCGTAAGCG	pQESLT-Q1484L
N1485A-1	CGCTTACGATCCTTATCA <u>gcT</u> GTTATGGGTGGAAC	pQESLT-N1485A
N1485A-2	GTTCCACCCATAACAgcTTGATAAGGATCGTAAGCG	pQESLT-N1485A
N1512A-1	CATTGGCTGCATAT <u>gcT</u> GCTGGGCCTGGTAAC	pQESLT-N1512A
N1512A-2	GTTACCAGGCCCAGCAgcATATGCAGCCAATG	pQESLT-N1512A
E1529Q-1	GGTATCCCTCCTTTTAAAc <u>AA</u> ACACAGAATTACGTC	pQESLT-E1529Q
E1529Q-2	GACGTAATTCTGTGTTTgTTTAAAAGGAGGGATAACC	pQESLT-E1529Q
H1580Q-1	CTCCGCTCCTCCCCA <u>CA</u> aAAAGGAACTGATTTTGC	pQEM23-H1580Q
H1580Q-2	GCAAAATCAGTTCCTTTtGTGTTGGGAGGAGCGGAG	pQEM23-H1580Q

D1584N-1	CCCCACACAAAGGAACT <u>a</u> ATTTTGGCTGCAAAAGCAG	pQEM23-D1584N
D1584N-2	CTGCTTTTGCAGCAAAATtAGTTCCTTTGTGTGGG	pQEM23-D1584N
N1612A-1	GCTACAGTAAAAGTGCAGGTgcCTGGGTTGTTATTAAACAGG	pQEM23-N1612A
N1612A-2	CCTGTTTAATAACAACCCAGgcACCTGCAGTTTTACTGTAGC	pQEM23-N1612A
H1628Q-1	GTTGCCAAGTACATGCAgATGCTTAACACTCCTTC	pQEM23-H1628Q
H1628Q-2	GAAGGAGTGTTAAGCATcTGCATGTACTTGGCAAC	pQEM23-H1628Q
Q1645A-1	GTCAATCAGTTAAAGCCGGTgcAACTATTGGTAAAGTTGGTAG	pQEM23-Q1645A
Q1645A-2	CTACCAACTTTACCAATAGTgcACCGGCTTTAACTGATTGAC	pQEM23-Q1645A
S1656A-1	GGTAGTACAGGGAACgCGACTGGGAACACCTTC	pQEM23-S1656A
S1656A-2	GAAGGTGGTTCCCAGTCGcGTTCCCTGTACTACC	pQEM23-S1656A
H1660Q-1	GGGAACTCGACTGGGAACCAgCTTCATTTACAGATCG	pQEM23-H1660Q
H1660Q-2	CGATCTGTAAATGAAGcTGGTCCCAGTCGAGTCCC	pQEM23-H1660Q
H1662Q-1	CTGGGAACCACCTTCAgTTACAGATCGAACAAAATGG	pQEM23-H1662Q
H1662Q-2	CCATTTTGTTCGATCTGTAAcTGAAGGTGGTTCCCAG	pQEM23-H1662Q
N1668A-1	CCTTCATTTACAGATCGAACAAgcTGGAAAAACAATCGATCCTG	pQEM23-N1668A
N1668A-2	CAGGATCGATTGTTTTTCCAgcTTGTTCGATCTGTAAATGAAGG	pQEM23-N1668A
D1673A-1	CAAAATGGAAAAACAATCGcTCCTGAAAAGTACATGCAAGG	pQEM23-D1673A
D1673A-2	CCTTGCATGTACTTTTCAGGAgCGATTGTTTTTCCATTTG	pQEM23-D1673A

Lowercase letters and underlining indicate mutated bases and mutated codons in CwIP-SLT and CwIP-M23, respectively. Lowercase letters shown in italics and double-underlining indicate tag sequences and restriction sites, respectively. Plasmids were constructed using the primers shown.

Supplemental Table S3. **Hydrolytic activities of CwIP-SLT, CwIP-M23 and their point-mutated proteins using zymography**

(A) CwIP-SLT

Protein name	WT	E1447Q	E1447A	S1448A	S1456A	Q1465A	D1481N	N1485A	N1512A	E1529Q
Relative activity	100	0.7	1.1	6.1	8.1	15.7	95.5	8.3	9.1	97.4

Aliquots of the wild-type CwIP-SLT [5 µg/ml (0.33 µM)] and the mutated CwIP-SLT were applied to gels and incubated at 37°C for 1 hour in renaturation solution (1% Triton X-100, pH 5.0). The hydrolytic activities were determined using the CS Analyzer 2.1 software (ATTO) and are shown along with the relative activity of the wild-type (WT) CwIP-SLT enzyme. Hydrolytic activity of less than 2% was within experimental error. Therefore, E1447Q and E1447A were determined to have no hydrolytic activity.

(B) CwIP-M23

Protein name	WT	H1580Q	D1584N	N1612A	H1628Q	Q1645A	S1656A	H1660Q	H1662Q	N1668A	D1673A
Relative activity	100	6.1	5.6	87.6	0.2	97.3	82.9	1.5	8.9	90.7	87.1

Aliquots of the wild-type CwIP-M23 [1 µg/ml (0.051 µM)] and the mutated CwIP-M23 were applied to gels and incubated at 40°C for 45 min in renaturation solution (1% Triton X-100, pH 7.5). The hydrolytic activities were determined using the CS Analyzer 2.1 software (ATTO) and are shown along with the relative activity of the wild-type (WT) CwIP-M23 enzyme. Hydrolytic activity of less than 2% was within experimental error. Therefore, H1628Q and H1660Q were determined to have no hydrolytic activity.