Science Advances
AAAS

Supplementary Materials for

Essential autoproteolysis of bacterial anti-σ factor RsgI for transmembrane signal transduction

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Sci. Adv. **9**, eadg4846 (2023)
DOI: 10.1126/sciadv.adg4846

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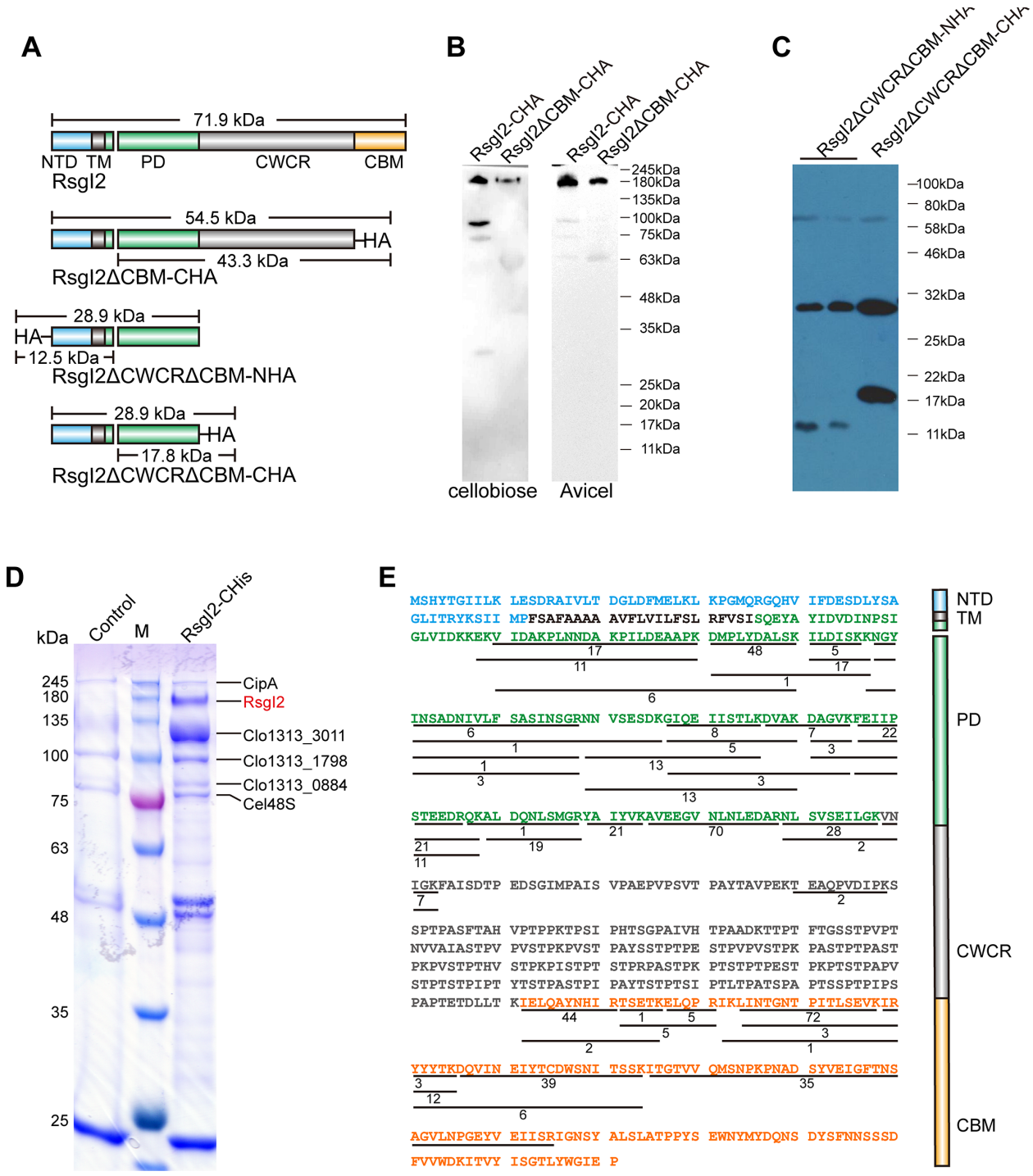

Fig. S1. Identification of RsgI2 expressed in *Clostridium thermocellum*. (**A**) Schematic representation of the composition of RsgI2 and its truncation mutants. (**B-C**) The expression of RsgI2 truncation mutants in *C. thermocellum*. The HA-tagged RsgI2 lacking the carbohydrate-binding domain (**B**) or lacking both the cell wall-crossing region (CWCR) and the CBM (**C**) were expressed in the Δ*rsgI2* strain with cellobiose or Avicel as the carbon source, and the RsgI components were detected by Western blotting using an anti-HA-tag antibody. (**D**) SDS-PAGE analysis of the purified RsgI2-CHis. The mutants ΔrsgI2 (control) and the ΔrsgI2 bearing a

plasmid to express RsgI2 with a C-terminal His tag (RsgI2-CHis) were cultured in media with Avicel as the carbon source. RsgI2-CHis was purified using the following procedure: After ultrasonication treatment of the cells, the lysate was centrifuged at 5,000 g, and the cell debris were dissolved in sample buffer (50 mM Tris-HCl, 500 mM NaCl, 30 mM imidazole, pH 8.0) containing 8 M urea. After ultracentrifugation at 160,000 g for 60 min, the pellet was dissolved in the sample buffer containing 1% n-dodecyl β-D-maltoside (DDM) and ultracentrifuged again at 160,000 g for 60 min. After the addition of 8 M urea, the supernatant fluids were applied to a Ni^{2+} affinity column. The proteins were eluted with the sample buffer containing 500 mM imidazole and 8 M urea. The eluted proteins were detected on SDS-PAGE, and the six designated protein bands in the range of 75-250 kDa were selected for mass spectroscopy identification. The identified proteins with the highest scores are shown in the figure. (**E**) The peptides identified by mass spectroscopy for the band at 180 kDa. Each detected peptide segment and its matching times are indicated.

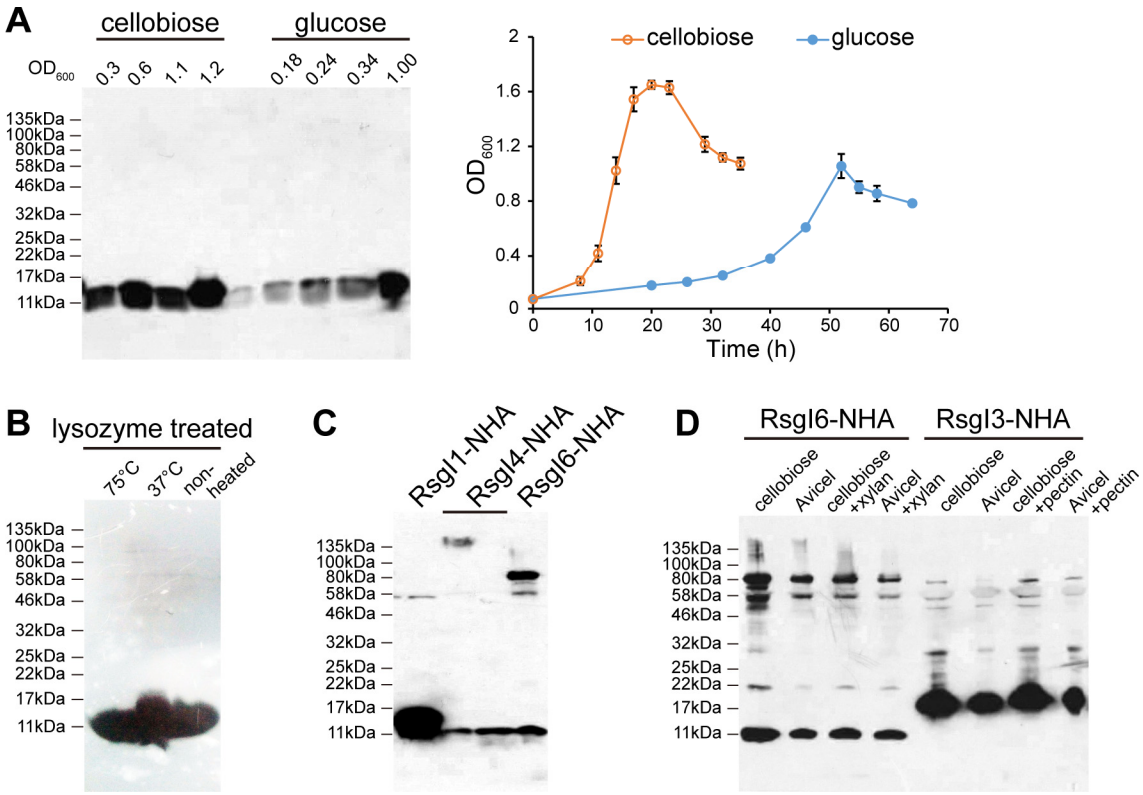

Fig. S2. Identification of the cleavage of different RsgIs in *C. thermocellum*. (**A**) The cleavage is independent of the carbon source and growth stage. The Δ*rsgI2*::*rsgI2-NHA* strain was cultured with cellobiose or glucose as the carbon source and sampled at different growth stages. Growth curves are shown on the right for reference. (**B**) Samples of Δ*rsgI2*::*rsgI2-NHA* cultured on cellobiose were lysed by lysozyme and then treated at different temperatures in a water bath. (**C**) The cleavages were observed in the N-terminal HA-tagged RsgIs expressed in *C. thermocellum* DSM1313 cultured with cellobiose as the carbon source. (**D**) The cleavage of RsgI6-NHA and RsgI3-NHA expressed in *C. thermocellum* with different carbon sources. All the targeted proteins were detected by Western blotting using an anti-HA-tag antibody.

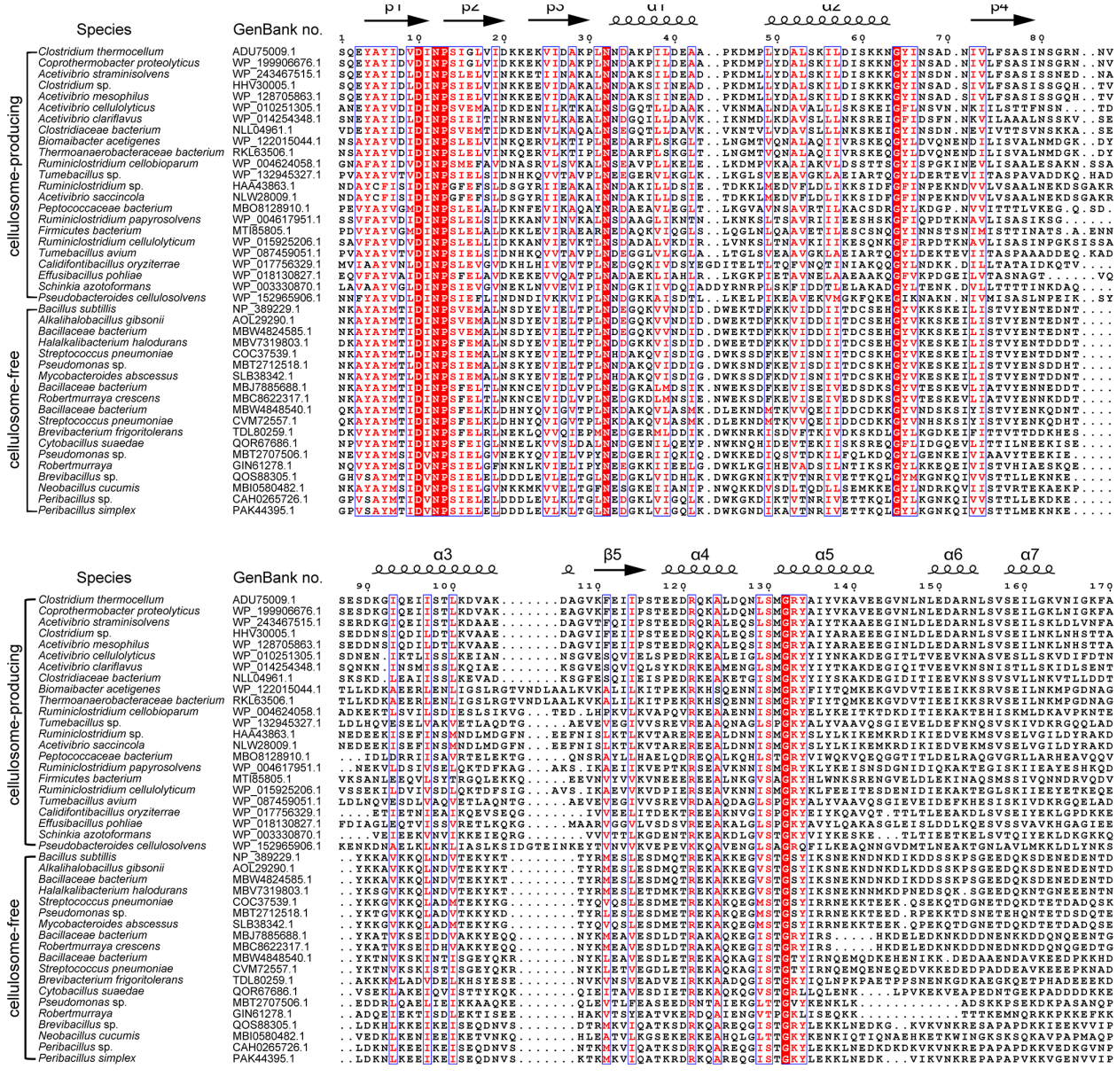

Fig. S3. Sequence alignments of the RsgI-PDs in cellulosome-producing and cellulosome-free bacteria. Secondary structure elements of RsgI2-PD are shown at the top of the alignment. The species names and GenBank numbers are shown on the left.

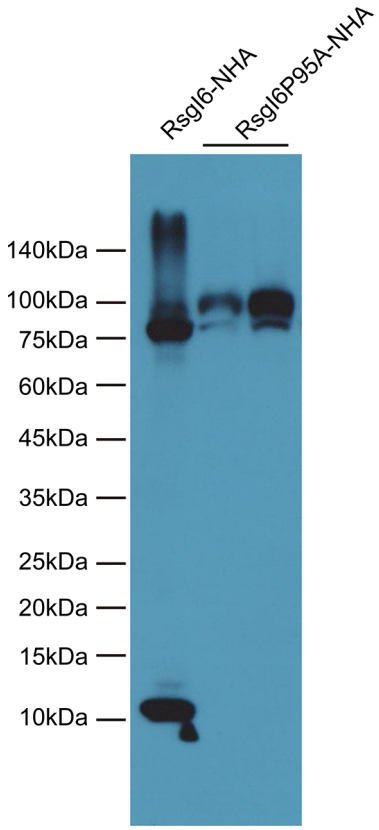

Fig. S4. The P95A mutation blocks the cleavage of RsgI6 in *C. thermocellum*.
DSM1313::*rsgI6-NHA* and DSM1313::*rsgI6P95A-NHA* were cultured with cellobiose as the carbon source and detected by Western-blot analysis using the anti-HA-tag antibody.

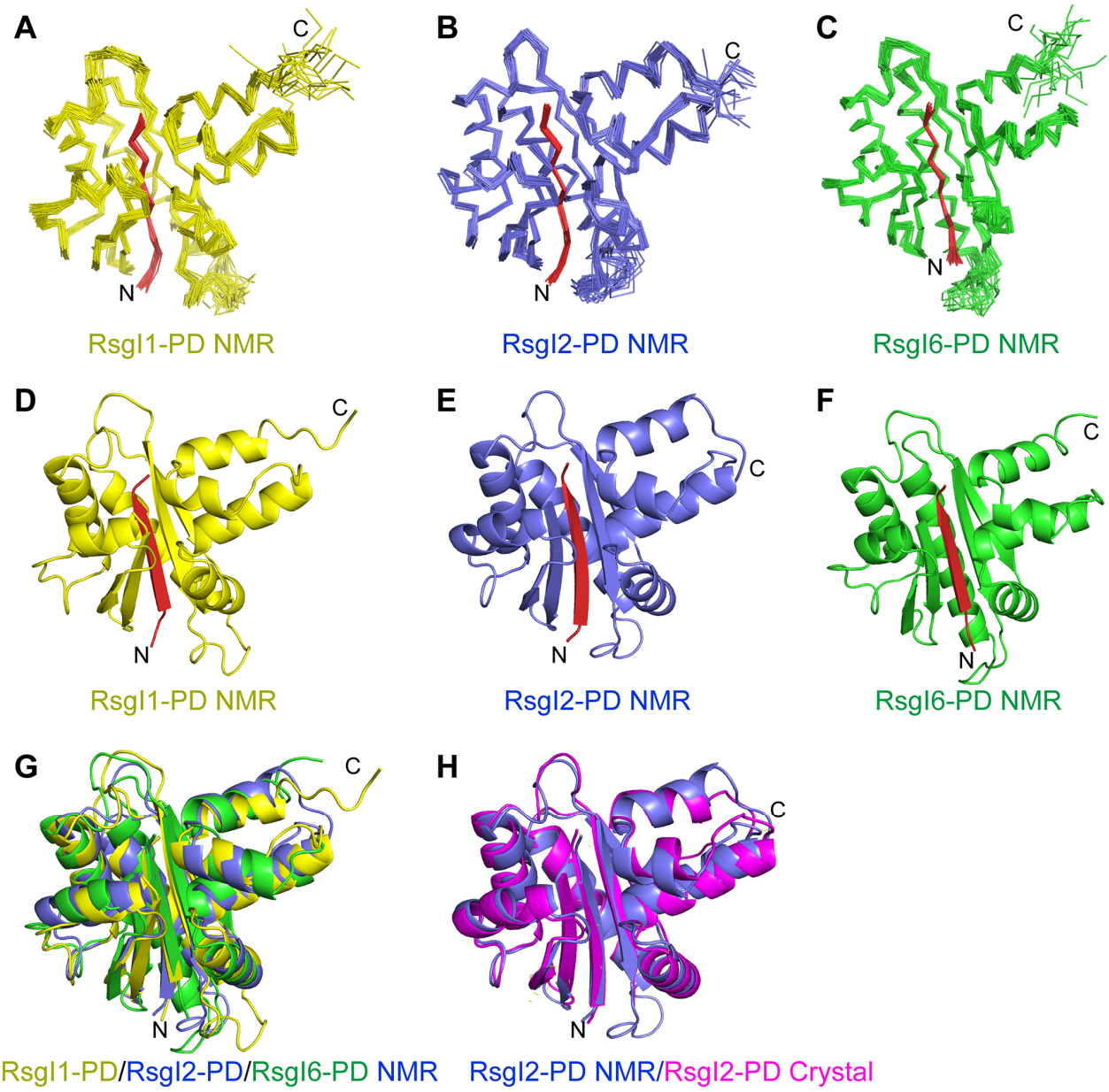

Fig. S5. NMR structures of RsgI-PDs. (**A-F**) The backbone ensemble of 20 structures (**A-C**) and ribbon representation of the first structure (**D-F**) of RsgI1-PD (**A** and **D**), RsgI2-PD (**B** and **E**), and RsgI6-PD (**C** and **F**). The disconnected β1 strands are shown in red in Panels **A-F**. (**G**) Superposition of the NMR structures of RsgI1-PD, RsgI2-PD, and RsgI6-PD. (**H**) Superposition of the NMR (blue) and crystal (purple) structures of RsgI2-PD.

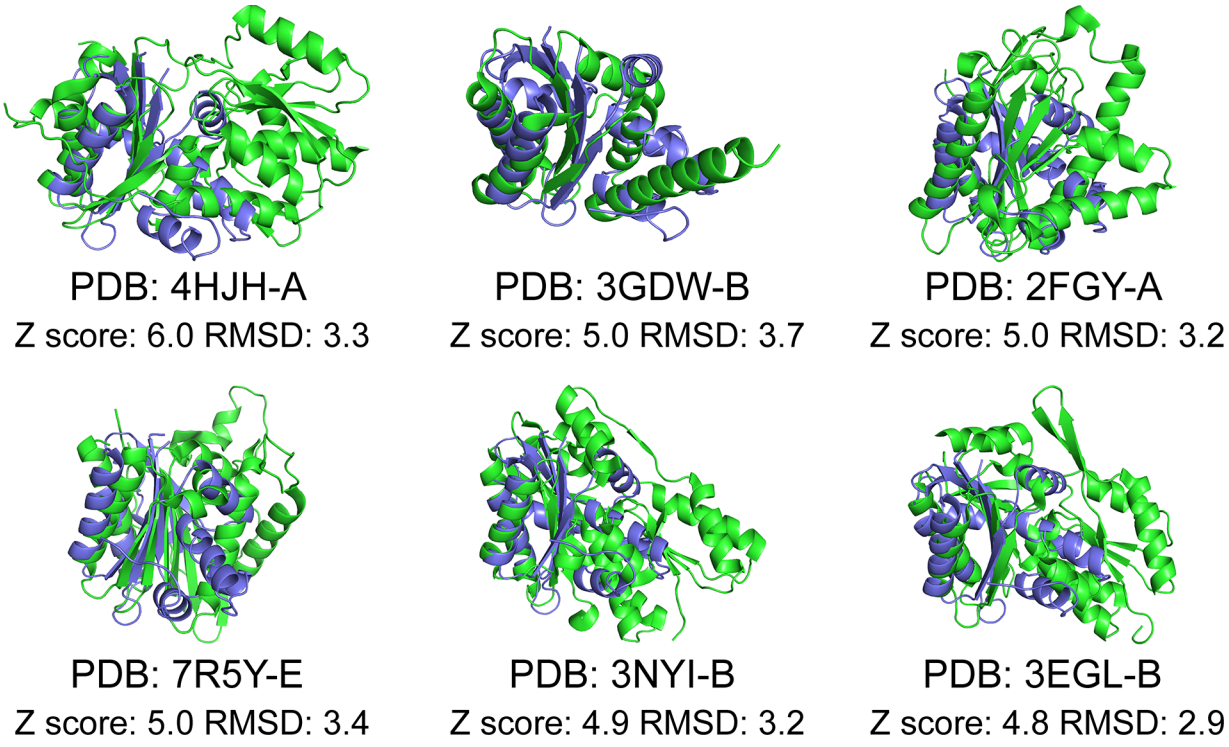

Fig. S6. Structure comparison of RsgI2-PD with similar structures identified by the Dali server. The six highest-scored structures and RsgI2-PD are shown in green and slate blue, respectively.

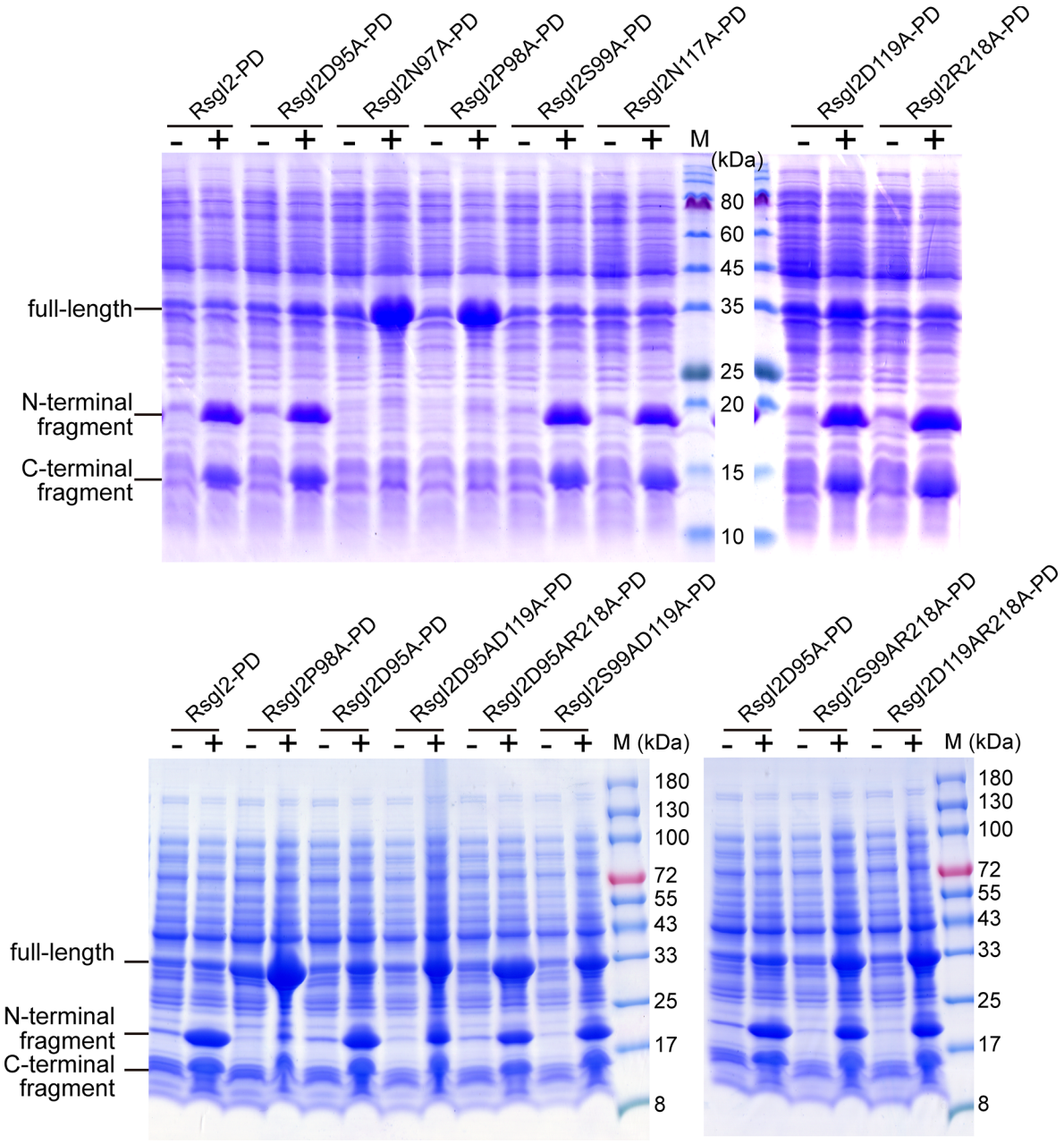

Fig. S7. Mutagenetic analysis of the conserved residues surrounding the cleavage site in SMT3-RsgI2-PD. The *Escherichia coli* BL21(DE3) cells expressing various mutants before (-) and after (+) induction were analyzed by SDS-PAGE.

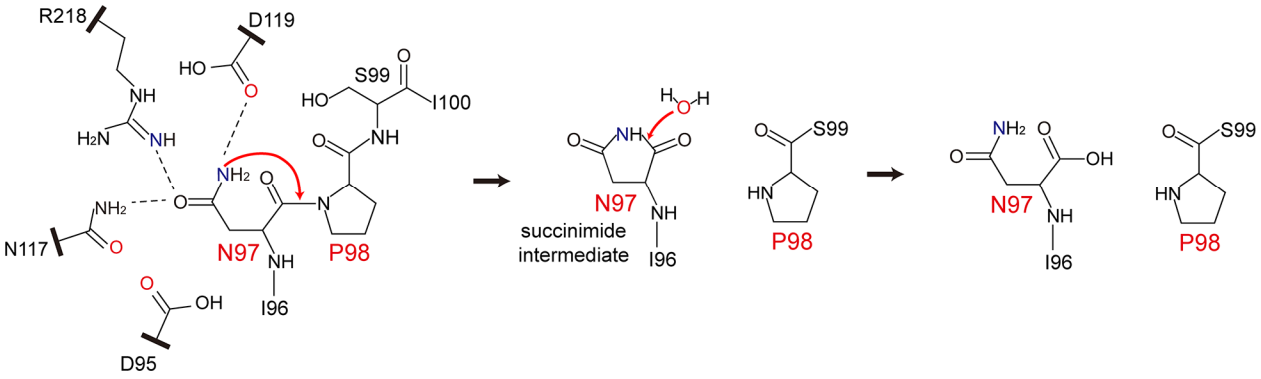

Fig. S8. Proposed autocleavage mechanism of RsgI2. Asn42 is polarized by one or more adjacent residues and the side chain performs a nucleophilic attack on its peptide bond, resulting in the autocleavage with a succinimide intermediate which is further opened by hydrolysis.

Xyn10Z (Clo1313_2635)

MSRKLFSVLL VGLMLMTSLL VTISSTSAAS LPTMPPSGYD QVRNGVPRGQ VVNISYFSTA TNTTRPARVY
LPPGYSKDKK YSVLYLLHGI GGSENDWFEG GGRANVIADN LIAEGKIKPL IIVTPNTNAA GPGIADGYEN
FTKDLLNSLI PYIESNYSVY TDREHRAIAG LSMGGGQSFN IGLTNLDKFA YIGPISAAPN TYPNERLFPD
GGKAAREKLK LLFIACGTND SLIGFGQRVH EYCVANNINH VYWLIQGGGH DFNVWKPGLW NFLQMADEAG
LTRDGNTPVP TPSPKPANTR IEAEDYDGIN SSSIEIIGVP PEGGRGIGYI TSGDYLVYKS IDFGNGATSF
KAKVANANTS NIELRLNGPN GTLIGTLSVK STGDWNTYEE QTCSISKVTG INDLYLVFKG PVNIDWFTFG
VESSSTGLGD LNGDGNINSS DLQALKRHLL GISPLTGEAL LRADVNRSGK VDSTDYSVLK RYILRIITEF
PGQGDVQTPN PSVTPTQTPI PTISGNALRD YAEARGIKIG TCVNYPFYNN SDPTYNSILQ REFSMVVCEN
EMKFDALQPR QNVFDFSKGD QLLAFAERNG MQMRGHTLIW HNQNPSWLTN GNWNRDSLLA VMKNHITTVM
THYKGKIVEW DVANECMDDS GNGLRSSIWR NVIGQDYLDY AFRYARFADP DALLFYNDYN IEDLGPKSNA
VFNMIKSMKE RGVPIDGVGF QCHFINGMSP EYLASIDQNI KRYAEIGVIV SFTEIDIRIP QSENPATAFQ
VQANNYKELM KICLANPNCN TFVMWGFTDK YTWIPGTFPG YGNPLIYDSN YNPKPAYNAI KEALMGY

Xyn11B (Clo1313_0522)

MKQKLLVTFL ILITFTVSLT LFPVNVRADV VITSNQTGTH GGYNFEYWKD TGNGTMVLKD GGAFSCEWSN
INNILFRKGF KYDETKTHDQ LGYITVTYSC NYQPNGNSYL GVYGWTSNPL VEYYIIESWG TWRPPGATPK
GTITVDGGTY EIYETTRVNQ PSIKGTATFQ QYWSVRTSKR TSGTISVTEH FKAWERLGMK MGKMYEVALV
VEGYQSSGKA DVTSMTITVG NAPSTSSPPG PTPEPTPRSA FSKIEAEEYN SLKSSTIQTI GTSDGGSGIG
YIESGDYLVF NKINFGNGAN SFKARVASGA DTPTNIQLRL GSPTGTLIGT LTVASTGGWN NYEEKSCSIT
NTTGQHDLYL VFSGPVNIDY FIFDSKGVNP TPTPTSPPQQ DQVLGDLNGD KQVNSTDYTA LKRHLLNITR
LSGTALANAD VNRDGKVDST DLMMLHRYLL RIISKLG

Fig. S9. Identification of the overexpressed Xyn10Z and Xyn11B in Δ*rsgI6*. The two additional bands in SDS-PAGE analysis (Fig. 4C) of the extracellular proteins of Δ*rsgI6* compared with wild-type strain were identified by LTQ-ESI-MS/MS mass spectrometry. The detected peptide segments are highlighted in green.

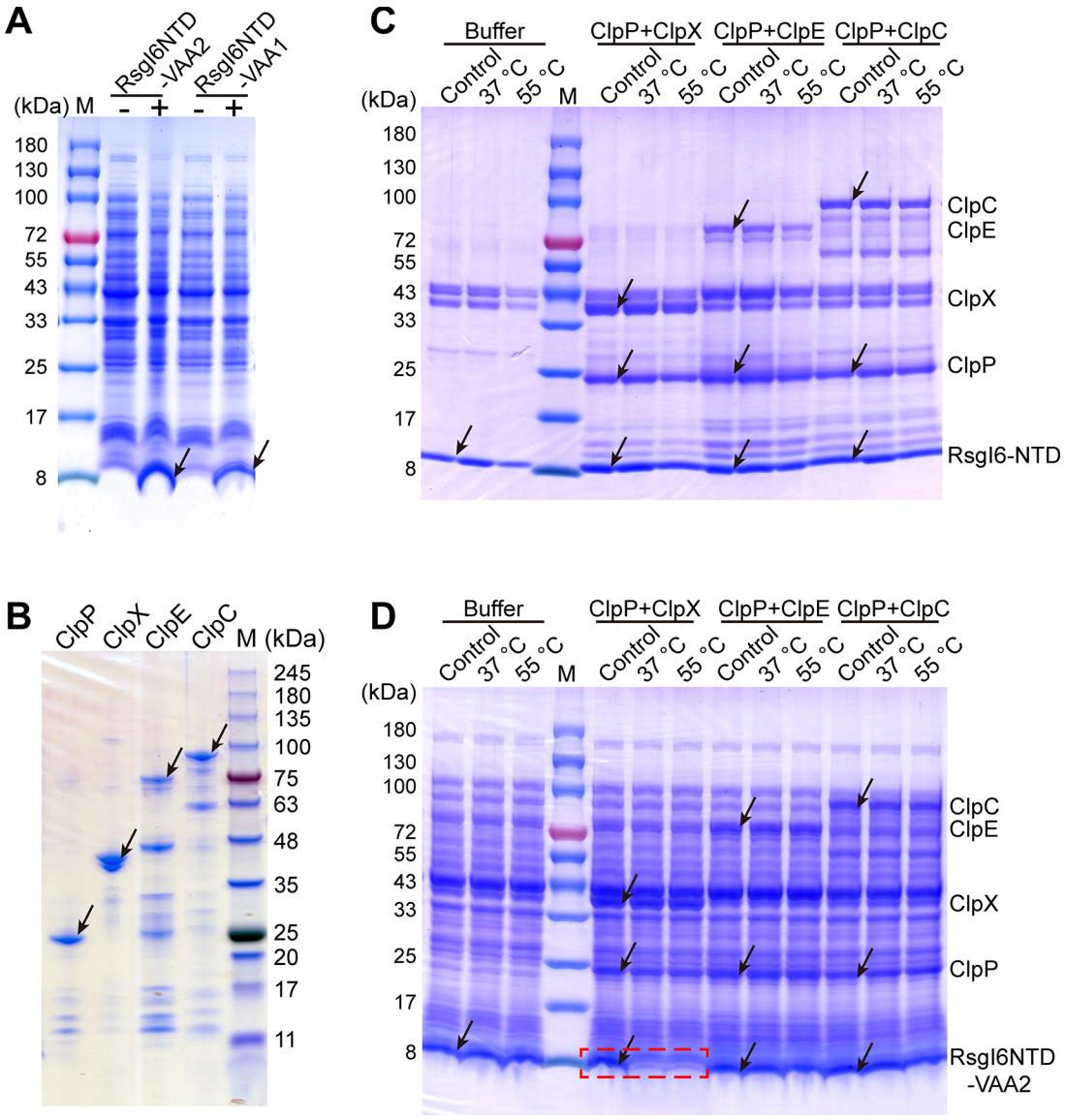

Fig. S10. Identification of the ClpP protease complex in the degradation of the RsgI6-NTD. (**A**) The cell lysate of overexpressed recombinant RgsI6NTD-VAA1 and RgsI6NTD-VAA2. (**B**) The purified recombinant ClpP, ClpX, ClpE, and ClpC. (**C-D**) The protease assays, using RsgI6NTD (**C**) and RsgI6NTD-VAA2 (**D**) as the substrate, respectively. The positions of the proteases and substrates in the control (the mixture before the reaction) are indicated by arrows. The degradation of substrate bands is indicated by a red-dashed rectangle.

Table S1.

Bacterial strains used in this study.

Strains	Relevant characteristic	Sources
E. coli		
DH5α	*f80dlacZΔM15*, *Δ(lacZYA-argF)U169*, *deoR*, *recA1*, *endA1*, *hsdR17*(r_k^-, m_k^+), *phoA*, *supE44*, l^-, *thi-1*, *gyrA96*, *relA1*	Transgen
BL21(DE3)	*ompT*, *gal*, *dcm*, *lon*, *hsdS*$_B$ (r_B^-, m_B^-), *l (DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])*	Transgen
BL21(DE3)::*pET28a-SMT3-rsgI2-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI2N97A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2N97A-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI2P98A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2P98A-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI2D95A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2D95A-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI2S99A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2S99A-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI2N117A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2N117A-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI2D119A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2D119A-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI2R218A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2R218A-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI2D95AD119A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2D95AD119A-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI2D95AR218A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2D95AR218A-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI2S99AD119A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2S99AD119A-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI2S99AR218A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2S99AR218A-PD expression	This work

BL21(DE3)::*pET28a-SMT3-rsgI2D119AR218A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI2D119AR218A-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI6-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI6-PD expression	This work
BL21(DE3)::*pET28a-SMT3-rsgI6P95A-PD*	BL21(DE3) strain containing a pET28a-SMT3 derivative for RsgI6P95A-PD expression	This work
BL21(DE3)::*pET28a-rsgI6-NTD*	BL21(DE3) strain containing a pET28a derivative for RsgI6-NTD expression	This work
BL21(DE3)::*pET28a-clpX*	BL21(DE3) strain containing a pET28a derivative for ClpX expression	This work
BL21(DE3)::*pET28a-clpE*	BL21(DE3) strain containing a pET28a derivative for ClpE expression	This work
BL21(DE3)::*pET28a-clpC*	BL21(DE3) strain containing a pET28a derivative for ClpC expression	This work
BL21(DE3)::*pET30a-rsgI1-PD*	BL21(DE3) strain containing a pET30a derivative for RsgI1-PD expression	This work
BL21(DE3)::*pET30a-rsgI2-PD*	BL21(DE3) strain containing a pET30a derivative for RsgI2-PD expression	This work
BL21(DE3)::*pET30a-rsgI6-PD*	BL21(DE3) strain containing a pET30a derivative for RsgI6-PD expression	This work
BL21(DE3)::*pET30a-rsgI6NTD-VAA1*	BL21(DE3) strain containing a pET30a derivative for RsgI6-NTD-VAA1 expression	This work
BL21(DE3)::*pET30a-rsgI6NTD-VAA2*	BL21(DE3) strain containing a pET30a derivative for RsgI6-NTD-VAA2 expression	This work
BL21(DE3)::*pET30a-clpP*	BL21(DE3) strain containing a pET30a derivative for ClpP expression	This work
C. thermocellum		
DSM1313	Wild type strain	DSMZ
Δ*rsgI2*	Derived from DSM1313, with deleted *rsgI2* gene *clo1313_1962*	This work
Δ*rsgI2*::*rsgI2-NHA*	Derived from Δ*rsgI2*, with the plasmid pHK-P_{2638}-RsgI2-NHA	This work
Δ*rsgI2*::*rsgI2P98A-NHA*	Derived from Δ*rsgI2*, with the plasmid pHK-P_{2638}-RsgI2P98A-NHA	This work
Δ*rsgI2*::*rsgI2-CHA*	Derived from Δ*rsgI2*, with the plasmid pHK-P_{2638}-RsgI2-CHA	This work
Δ*rsgI2*::*rsgI2-CHis*	Derived from Δ*rsgI2*, with the plasmid pHK-P_{2638}-RsgI2-CHis	This work
Δ*rsgI2*::*rsgI2*Δ*CBM-CHA*	Derived from Δ*rsgI2*, with the plasmid pHK-P_{2638}-RsgI2ΔCBM-CHA	This work
Δ*rsgI2*::*rsgI2*Δ*CWCR*Δ*CBM-CHA*	Derived from Δ*rsgI2*, with the plasmid pHK-P_{2638}-RsgI2ΔCWCRΔCBM-CHA	This work
Δ*rsgI2*::*rsgI2*Δ*CWCR*Δ*CBM-NHA*	Derived from Δ*rsgI2*, with the plasmid pHK-P_{2638}-RsgI2ΔCWCRΔCBM-NHA	This work

DSM1313::*rsgI1-NHA*	Derived from DSM1313, with the plasmid pHK-P_{2638}-RsgI1NHA	This work
DSM1313::*rsgI3-NHA*	Derived from DSM1313, with the plasmid pHK-P_{2638}-RsgI3NHA	This work
DSM1313::*rsgI4-NHA*	Derived from DSM1313, with the plasmid pHK-P_{2638}-RsgI4NHA	This work
DSM1313::*rsgI6-NHA*	Derived from DSM1313, with the plasmid pHK-P_{2638}-RsgI6NHA	This work
DSM1313::*rsgI6P95A-NHA*	Derived from DSM1313, with the plasmid pHK-P_{2638}-RsgI6P95A-NHA	This work
DSM1313::*rsgI8-NHA*	Derived from DSM1313, with the plasmid pHK-P_{2638}-RsgI8NHA	This work
Δ*pyrF*	Derived from DSM1313, with deleted *pyrF* gene *clo1313_1266*	(48)
Δ*rsgI6*	Derived from Δ*pyrF*, with deleted *rsgI6* gene *clo1313_2777*	This work
Δ*rsgI6*::*rsgI6*	Derived from Δ*rsgI6*, with the plasmid pHK-P_{sigI6}-RsgI6	This work
Δ*rsgI6*::*rsgI6-T1*	Derived from Δ*rsgI6*, with the plasmid pHK-P_{sigI6}-RsgI6-T1	This work
Δ*rsgI6*::*rsgI6-T2*	Derived from Δ*rsgI6*, with the plasmid pHK-P_{sigI6}-RsgI6-T2	This work
Δ*rsgI6*::*rsgI6-T3*	Derived from Δ*rsgI6*, with the plasmid pHK-P_{sigI6}-RsgI6-T3	This work
Δ*rsgI6*::*rsgI6-T4*	Derived from Δ*rsgI6*, with the plasmid pHK-P_{sigI6}-RsgI6-T4	This work
Δ*rsgI6*::*rsgI6-T5*	Derived from Δ*rsgI6*, with the plasmid pHK-P_{sigI6}-RsgI6-T5	This work
Δ*rsgI6*Δ*rseP*	Derived from Δ*pyrF*, with deleted *rsgI6* gene *clo1313_2777* and *rsep* gene *clo1313_1217*	This work
Δ*rsgI6*Δ*rseP*::*rsgI6-T3*	Derived from Δ*rsgI6*Δ*rsep*, with the plasmid pHK-P_{sigI6}-RsgI6-T3	This work
Δ*sigI6-rsgI6*	Derived from Δ*pyrF*, with deleted *sigI6* and *rsgI6* gene *clo1313_2778* and *clo1313_2777*	This work
Δ*sigI6-rsgI6*::*sigI6-rsgI6*	Derived from Δ*sigI6-rsgI6*, with the plasmid pHK-P_{sigI6}-SigI6-RsgI6	This work
Δ*sigI6-rsgI6*::*sigI6-rsgI6P95A*	Derived from Δ*sigI6-rsgI6*, with the plasmid pHK-P_{sigI6}-SigI6-RsgI6-P95A	This work
Δ*sigI6-rsgI6*::*sigI6-rsgI6N94AP95A*	Derived from Δ*sigI6-rsgI6*, with the plasmid pHK-P_{sigI6}-SigI6-RsgI6-N94AP95A	This work

Table S2.

Plasmids used in this study.

Plasmids	Relevant characteristic
pET28a-SMT3-RsgI2-PD	pET28a derivative for RsgI2-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2N97A-PD	pET28a derivative for RsgI2N97A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2P98A-PD	pET28a derivative for RsgI2P98A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2D95A-PD	pET28a derivative for RsgI2D95A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2S99A-PD	pET28a derivative for RsgI2S99A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2N117A-PD	pET28a derivative for RsgI2N117A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2D119A-PD	pET28a derivative for RsgI2D119A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2R218A-PD	pET28a derivative for RsgI2R218A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2D95AD119A-PD	pET28a derivative for RsgI2D95AD119A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2D95AR218A-PD	pET28a derivative for RsgI2D95AR218A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2S99AD119A-PD	pET28a derivative for RsgI2S99AD119A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2S99AR218A-PD	pET28a derivative for RsgI2S99AR218A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI2D119AR218A-PD	pET28a derivative for RsgI2D119AR218A-PD (residues 86-259) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-SMT3-RsgI6-PD	pET28a derivative for RsgI6-PD (residues 84-258) expression in BL21(DE3) with N-terminal His tag and SMT3 tag

pET28a-SMT3-RsgI6P95A-PD	pET28a derivative for RsgI6P95A-PD (residues 84-258) expression in BL21(DE3) with N-terminal His tag and SMT3 tag
pET28a-RsgI6-NTD	pET28a derivative for RsgI6-NTD (residues 1-50) expression in BL21(DE3) with N-terminal His tag
pET28a-ClpX	pET28a derivative for ClpX (residues 61-409) expression in BL21(DE3) with N-terminal His tag
pET28a-ClpE	pET28a derivative for ClpE (residues 138-779) expression in BL21(DE3) with N-terminal His tag
pET28a-ClpC	pET28a derivative for ClpC expression in BL21(DE3) with N-terminal His tag
pET30a-RsgI2-PD	pET30a derivative for RsgI2PD (residues 89-248) expression in BL21(DE3) with C-terminal His tag
pET30a-RsgI1-PD	pET30a derivative for RsgI1PD (residues 87-253) expression in BL21(DE3) with C-terminal His tag
pET30a-RsgI6-PD	pET30a derivative for RsgI6PD (residues 86-252) expression in BL21(DE3) with C-terminal His tag
pET30a-RsgI6-NTD-VAA1	pET30a derivative for RsgI6-NTD-VAA1 (residues 1-60) expression in BL21(DE3) without His tag
pET30a-RsgI6-NTD-VAA2	pET30a derivative for RsgI6-NTD-VAA2 (residues 1-63) expression in BL21(DE3) without His tag
pET30a-ClpP	pET30a derivative for full-length ClpP expression in BL21(DE3) with C-terminal His tag
pLin-1d-GST-RsgI2-PD-Flag	pLin-1d derivative for RsgI2PD (residues 89-248) expression in the cell-free PURE system with N-terminal GST tag and C-terminal Flag tag
pHK-TT1A-RsgI2 9a	pHK-TT1A derivative, RsgI2 9a intron
pHK-TT1A-RseP 13a	pHK-TT1A derivative, RseP 13a intron
pHK-HR-RsgI6	pHK-HR derivative, containing *rsgI6* gene upstream and downstream sequence
pHK-HR-SigI-RsgI6	pHK-HR derivative, containing *sigI6-rsgI6* gene upstream and downstream sequence
pHK-P_{2638}-RsgI2-CHA	pHK derivative for RsgI2 expression in *C. thermocellum* driven by the *clo1313_2638* promoter with C-terminal HA tag
pHK-P_{2638}-RsgI2-NHA	pHK derivative for RsgI2 expression in *C. thermocellum* driven by the *clo1313_2638* promoter with N-terminal HA tag
pHK-P_{2638}-RsgI2P98A-NHA	pHK derivative for RsgI2P98A expression in *C. thermocellum* driven by the *clo1313_2638* promoter with N-terminal HA tag
pHK-P_{2638}-RsgI2-CHis	pHK derivative for RsgI2 expression in *C. thermocellum* driven by the *clo1313_2638* promoter with C-terminal His tag
pHK-P_{2638}-RsgI2ΔCBM-CHA	pHK derivative for RsgI2ΔCBM (deletes the C-terminal CBM3b domain, residues 1-504) expression in *C.*

	thermocellum driven by the *clo1313_2638* promoter with C-terminal HA tag
pHK-*P*$_{2638}$-RsgI2ΔCWCRΔCBM-CHA	pHK derivative for RsgI2ΔCWCRΔCBM (deletes the cell wall-crossing region and the C-terminal CBM3b domain, residues 1-248) expression in *C. thermocellum* driven by the *clo1313_2638* promoter with C-terminal HA tag
pHK-*P*$_{2638}$-RsgI2ΔCWCRΔCBM-NHA	pHK derivative for RsgI2ΔCWCRΔCBM (deletes the cell wall-crossing region and the C-terminal CBM3b domain, residues 1-248) expression in *C. thermocellum* driven by the *clo1313_2638* promoter with N-terminal HA tag
pHK-*P*$_{2638}$-RsgI1-NHA	pHK derivative for RsgI1 expression in *C. thermocellum* driven by the *clo1313_2638* promoter with N-terminal HA tag
pHK-*P*$_{2638}$-RsgI3-NHA	pHK derivative for RsgI3 expression in *C. thermocellum* driven by the *clo1313_2638* promoter with N-terminal HA tag
pHK-*P*$_{2638}$-RsgI4-NHA	pHK derivative for RsgI4 expression in *C. thermocellum* driven by the *clo1313_2638* promoter with N-terminal HA tag
pHK-*P*$_{2638}$-RsgI6-NHA	pHK derivative for RsgI6 expression in *C. thermocellum* driven by the *clo1313_2638* promoter with N-terminal HA tag
pHK-*P*$_{2638}$-RsgI6P95A-NHA	pHK derivative for RsgI6P95A expression in *C. thermocellum* driven by the *clo1313_2638* promoter with N-terminal HA tag
pHK-*P*$_{2638}$-RsgI8-NHA	pHK derivative for RsgI8 expression in *C. thermocellum* driven by the *clo1313_2638* promoter with N-terminal HA tag
pHK-*P*$_{sigI6}$-RsgI6	pHK derivative for RsgI6 expression in *C. thermocellum* driven by *sigI6* (*clo1313_2778*) promoter
pHK-*P*$_{sigI6}$-RsgI6-T1	pHK derivative for RsgI6-T1 (contains the N-terminal cytoplasmic domain, residues 1-50) expression in *C. thermocellum* driven by *sigI6* promoter
pHK-*P*$_{sigI6}$-RsgI6-T2	pHK derivative for RsgI6-T2 (contains the N-terminal domain and the transmembrane helix, residues 1-83) expression in *C. thermocellum* driven by *sigI6* promoter
pHK-*P*$_{sigI6}$-RsgI6-T3	pHK derivative for RsgI6-T3 (contains the N-terminal domain, the transmembrane helix, and β1 of the periplasmic domain, residues 1-94) expression in *C. thermocellum* driven by *sigI6* promoter
pHK-*P*$_{sigI6}$-RsgI6-T4	pHK derivative for RsgI6-T4 (deletion of the cell wall-crossing region and the C-terminal GH5 domain, residues 1-252) expression in *C. thermocellum* driven by *sigI6* promoter

pHK-P_{sigI6}-RsgI6-T5	pHK derivative for RsgI6-T5 (deletion of the C-terminal GH5 domain, residues 1-455) expression in *C. thermocellum* driven by *sigI6* promoter
pHK-P_{sigI6}-SigI6-RsgI6	pHK derivative for SigI6-RsgI6 expression in *C. thermocellum* driven by *sigI6* promoter
pHK-P_{sigI6}-SigI6-RsgI6P95A	pHK derivative for SigI6-RsgI6P95A expression in *C. thermocellum* driven by *sigI6* promoter
pHK-P_{sigI6}-SigI6-RsgI6N94AP95A	pHK derivative for SigI6-RsgI6N94AP95A expression in *C. thermocellum* driven by the *sigI6* promoter

Table S3.

Primers used in this study.

Primers	Sequences (5'-3')[a]	Notes
RsgI2-PD$^{86-259}$-F	TTAGGATCCTCCCAGGAATATGCTTATATAGATG	To construct the RsgI-PDs expression plasmids in *E. coli*
RsgI2-PD$^{86-259}$-R	ATTACTCGAGTCAAGTATCGGAGATGGCG	
RsgI1-PD$^{87-253}$-F	CGCCATATGTACGGATATATATGCGTTG	
RsgI1-PD$^{87-253}$-R	CCGCTCGAGTTTCTCTATCATATCTGAAATAC	
RsgI6-PD$^{86-252}$-F	CGCCATATGTATGCTTATGTTGGCATTG	
RsgI6-PD$^{86-252}$-R	CCGCTCGAGCTTGGCAATGAGCTCCAG	
RsgI2-PD$^{89-248}$-F	TAACATATGTATGCTTATATAGATGTTGATATAAAT	
RsgI2-PD$^{89-248}$-R	TTTACTCGAGTTTGCCAAGAATCTCGG	
N97A-F	CTTATATAGATGTTGATATAGCTCCAAGCATTGGGCTTGTAAT	For the mutation of RsgI2
N97A-R	ATTACAAGCCCAATGCTTGGAGCTATATCAACATCTATATAAG	
P98A-F	ATATAGATGTTGATATAAATGCTAGCATTGGGCTTGTAATTGA	
P98A-R	TCAATTACAAGCCCAATGCTAGCATTTATATCAACATCTATAT	
D95A-F	AATATGCTTATATAGATGTTGCTATAAATCCAAGCATTGGGCT	
D95A-R	AGCCCAATGCTTGGATTTATAGCAACATCTATATAAGCATATT	
S99A-F	TAGATGTTGATATAAATCCAGCTATTGGGCTTGTAATTGATAA	
S99A-R	TTATCAATTACAAGCCCAATAGCTGGATTTATATCAACATCTA	
N117A-F	TTATAGACGCAAAACCTTTAGCTAATGACGCAAAACCGATTCT	
N117A-R	AGAATCGGTTTTGCGTCATTAGCTAAAGGTTTTGCGTCTATAA	
D119A-F	ACGCAAAACCTTTAAATAATGCTGCAAAACCGATTCTCGATGA	
D119A-R	TCATCGAGAATCGGTTTTGCAGCATTATTTAAAGGTTTTGCGT	
R218A-F	ACCAAAATCTTTCCATGGGAGCTTATGCTATATATGTAAAAGC	

R218A-R	GCTTTTACATATATAGCATAAGCTCC CATGGAAAGATTTTGGT	
P95A-F	TATGTTGGCATTGATATAAATGCTAG TATTGAGCTTTGGATAAAT	For the mutation of RsgI6
P95A-R	ATTTATCCAAAGCTCAATACTAGCA TTTATATCAATGCCAACATA	
N94AP95A-F	TATGTTGGCATTGATATAGCTGCTAG TATTGAGCTTTGGATAAAT	
N94AP95A-R	ATTTATCCAAAGCTCAATACTAGCA GCTATATCAATGCCAACATA	
RsgI2 9a IBS12	AAAACTAGTAAtgattcccgtgtaGTGCG ACGCGAAAGCTAG	To construct the corresponding targetrons
RsgI2 9a EBS2	CGCTAGAAGCCTCGTTAaatcaAGCA GGCCAAAGATGCTG	
RsgI2 9a EBS1a	CCCCGTACGCTGAcgtgtaAGCAGCGt ATCCAATCC	
RseP 13a IBS12	AAAACTAGTAAgccagaataaccaGTGCG ACGCGAAAGCTAG	
RseP 13a EBS2	CGCTAGAAGCCTCGTTActggcAGCA GGCCAAAGATGCTG	
RseP 13a EBS1a	CCCCGTACGCTGAtaaccaAGCAGCGt ATCCAATCC	
RsgI6_up-F	CCGGAATTCGCCATGGAAAATCCC GATG	For the construction of Δ*rsgI6* by homologous recombination
RsgI6_up-R	ACGCGTCGACCGGTATTTTGAATGC GGGAA	
RsgI6_m-F	CCGCTCGAGAAGTCCTGGTTGAGC ACAAA	
RsgI6_m-R	GAAAAGGAGGTGGATTTGCGCGGT ATTTTGAATGCGGGAA	
RsgI6_down-F	TTCCCGCATTCAAAATACCGCGCAA ATCCACCTCCTTTTC	
RsgI6_down-R	CCGAGATCTATTTGTTTGCACCTGT TTGA	
SigI6-RsgI6_up-F	CCGGAATTCGCCATGGAAAATCCC GATG	For the construction of Δ*sigI6-rsgI6* by homologous recombination
SigI6-RsgI6_up-R	ACGCGTCGACCGGTATTTTGAATGC GGGAA	
SigI6-RsgI6_m-F	CCGCTCGAGAAGTCCTGGTTGAGC ACAAA	
SigI6-RsgI6_m-R	GAATAAGGGGTGAAATTAACCGCC GGTATTTTGAATGCGGGAA	
SigI6-RsgI6_down-F	TTCCCGCATTCAAAATACCGGCGGT TAATTTCACCCCTTATTC	
SigI6-RsgI6_down-R	CCGAGATCTTCCATAATTTCATTAC CAAT	

RsgI2-CHA-F	CCGGAATTCTCACATTACACGGGAATCAT	To construct the overexpression plasmids of RsgIs with an HA tag or His tag in *C. thermocellum*
RsgI2-CHA-R	CCGCTCGAGTTA*AGCGTAATCCGGTACATCGTACGGGTA*ATTATTAGGTTCAATTCCCCAATATAG	
RsgI2-NHA-F	CCGGAATTC*TACCCGTACGATGTACCGGATTACGCT*AATAATTCACATTACACGGGAATCAT	
RsgI2-NHA-R	CCGCTCGAGTTAAGGTTCAATTCCCCAAT	
RsgI2-CHis-F	CCGGAATTCTCACATTACACGGGAATCAT	
RsgI2-CHis-R	CCGCTCGAGTTA*ATGGTGATGGTGATGATG*TGATCCAGGTTCAATTCCCCAAT	
RsgI2ΔCBM-CHA-F	CCGGAATTCTCACATTACACGGGAATCAT	
RsgI2ΔCBM-CHA-R	CCGCTCGAGTTA*AGCGTAATCCGGTACATCGTACGGGTA*ATTATTCGTAGGCGCTGGCGATGGA	
RsgI2ΔCWCRΔCBM-CHA-F	CCGGAATTCTCACATTACACGGGAATCAT	
RsgI2ΔCWCRΔCBM-CHA-R	CCGCTCGAGTTA*AGCGTAATCCGGTACATCGTACGGGTA*ATTATTTACTTTGCCAAGAATCTCGG	
RsgI2ΔCWCRΔCBM-NHA-F	CCGGAATTC*TACCCGTACGATGTACCGGATTACGCT*AATAATTCACATTACACGGGAATCAT	
RsgI2ΔCWCRΔCBM-NHA-R	CCGCTCGAGTTATACTTTGCCAAGAATCTCGG	
RsgI1-NHA-F	CCGGAATTC*TACCCGTACGATGTACCGGATTACGCT*AATAATAACAGATTGGGAATAATATATG	
RsgI1-NHA-R	CCGCTCGAGTTATCAATTGGGCTCAATTCC	
RsgI3-NHA-F	CCGGAATTC*TACCCGTACGATGTACCGGATTACGCT*AATAATGATAACATAGGAGTAATCATTAAG	
RsgI3-NHA-R	CCGCTCGAGTTAATCTGCAAACAAATTTTTTG	
RsgI4-NHA-F	CCGGAATTC*TACCCGTACGATGTACCGGATTACGCT*AATAATAATCTTGGAGTGGTAATAAAAA	
RsgI4-NHA-R	CCGCTCGAGTCATGGTTCTCTTCCCCAT	

RsgI6-NHA-F	CCGGAATTC*TACCCGTACGATGTACC GGATTACGCT*AATAATATTGTAGGAA AAGTTCTTGATAT	
RsgI6-NHA-R	CCGCTCGAGTCAGGGAATTCTGTA AGTAGTC	
RsgI8-NHA-F	CCGGAATTC*TACCCGTACGATGTACC GGATTACGCT*AATAATACAAAACAA AAAGGTACTA	
RsgI8-NHA-R	CCGCTCGAGCTATGGCATTTTTTCA ATG	
P_{sigI6}-F	**TATGCTTCCGGCTCGTATGT**AAG ATATTTGGGTCATAC	To construct the plasmids for expression of the truncations of RsgI6 in Δ*rsgI6* and SigI6-RsgI6 in Δ*sigI6-rsgI6*
P_{sigI6}-R	**CCGGTACATCGTACGGGTA**CATC GGTTAATTTCACCCC	
RsgI6-F	**ATAAGGGGTGAAATTAACCG**GTG ATTGTAGGAAAAGTTCT	
RsgI6-R	**TATTTTTATTTCTAGACTCGAG**TC AGGGAATTCTGTAAGTAG	
RsgI6-T5-R	**TATTTTTATTTCTAGACTCGAG**TC AGGATTCATTTTCAAACACAG	
RsgI6-T4-R	**TATTTTTATTTCTAGACTCGAG**TC ACTTGGCAATGAGCTCCAG	
RsgI6-T3-R	**TATTTTTATTTCTAGACTCGAG**TC AATTTATATCAATGCCAACATAAGC	
RsgI6-T2-R	**TATTTTTATTTCTAGACTCGAG**TC ATTTTCTTGCCGTATTTCC	
RsgI6-T1-R	**TATTTTTATTTCTAGACTCGAG**TC AATTTTTAGGCTTGATTA	
SigI6-RsgI6-F	**ATAAGGGGTGAAATTAACCG**GTG GATTGGCATTTTCAAGG	
SigI6-RsgI6-R	**TATTTTTATTTCTAGACTCGAG**TC AGGGAATTCTGTAAGTAG	
SigI6-F	GGGATGATTCTGCAAGGGAAGA	For qRT-PCR
SigI6-R	TGCCTGTCAGTCGCCTTATACA	
Xyn10Z-F	TCTTACACGGCATAGGCGGTAG	
Xyn10Z-R	CGGCAATCAGATTGTCGGCAAT	
Xyn11B-F	ACCAGCAATCCGCTTGTAGAGT	
Xyn11B-R	CTCGTATGTACCACCGTCAACG	
Xyn10D-F	CGGACAGGACTCAGGATTGGAA	
Xyn10D-R	AACTTTGACCCATGCCGAAACC	
Cel9K-F	CCGTGGCATACTTGCGAAGACA	
Cel9K-R	AGCTTTGTTCCCAGGCTCTCCT	
RPS-F	TGCCGTTGCCTACAGAAAGA	
RPS-R	GCGTCTACCGTCTTTGGTGTAG	
RsgI6-NTD-F	**TGCCGCGCGGCAGCCATATG**ATT GTAGGAAAAGTTCTTGAT	For construct the plasmids for

RsgI6-NTD-R	**TGGTGGTGGTGGTGCTCGAG**TCA ATTTTTAGGCTTGATTA	expression of proteins used in ClpP protease assay
RsgI6-NTD-VAA1-F	**AAGAAGGAGATATACATATG**ATTG TAGGAAAAGTTCTTGAT	
RsgI6-NTD-VAA1-R	**TGGTGGTGGTGGTGCTCGAG**TCA TGCCGCAACCGGCAAATATC	
RsgI6-NTD-VAA2-F	**AAGAAGGAGATATACATATG**ATTG TAGGAAAAGTTCTTGAT	
RsgI6-NTD-VAA2-R	**TGGTGGTGGTGGTGCTCGAG**TCA TGCAGCAACTGCCGCAACCG	
ClpP-F	**AAGAAGGAGATATACATATG**AGTT TGGTACCGATAGT	
ClpP-R	**TGGTGGTGGTGGTGCTCGAG**TTT TCTTCTTTCCATAACTTCG	
ClpX-F	**TGCCGCGCGGCAGCCATATG**ATA CCAAAGCCCAGTGAAAT	
ClpX-R	**TGGTGGTGGTGGTGCTCGAG**TCA ATTTATTATTACAGTCGGTGG	
ClpE-F	**TGCCGCGCGGCAGCCATATG**CCG AAGAAGAAAAAGTATCT	
ClpE-R	**TGGTGGTGGTGGTGCTCGAG**TCA CGGTTTTTCCACATTAAACA	
ClpC-F	**TGCCGCGCGGCAGCCATATG**TAC GGACGTTTTACCGAAAAAG	
ClpC-R	**TGGTGGTGGTGGTGCTCGAG**TTA GCCCTTGTTTGAAACAAG	

[a] Restriction sites are underlined, and mutation sites are colored in red. Sequences of homology segments for seamless cloning are shown in bold. The targeted sequences in the Thermotargetron primers are indicated by lowercase letters. The HA or His tag is indicated by italics.

Table S4.

NMR structural statistics of RsgI-PD.

Parameters	RsgI1-PD (PDB 8HEP)	RsgI2-PD (PDB 8HEQ)	RsgI6-PD (PDB 8HER)
NOE restraints			
Intra-residue	1190	1266	1496
Sequential	767	826	903
Medium-range	423	624	600
Long-range	614	809	799
Ambiguous	1992	2412	2068
Total	4986	5837	5866
Number per residue	28.32	35.13	33.32
Hydrogen bond restraints	170	168	178
Torsion angle restraints			
Phi (Φ) angle restraints	149	146	155
Psi (Ψ) angle restraints	149	146	155
Violations			
Max. NOE violation (Å)	0.185	0.196	0.193
Max. torsion angle violation (°)	3.695	3.201	4.776
R.M.S.D from mean structure (Å)			
Backbone in regular secondary structure	0.45 ± 0.07 [a]	0.38 ± 0.05 [b]	0.37 ± 0.06 [c]
Heavy atoms in regular secondary structure	0.81 ± 0.06 [a]	0.75 ± 0.04 [b]	0.70 ± 0.04 [c]
Backbone for all residues [d]	0.63 ± 0.07	0.56 ± 0.09	0.69 ± 0.14
Heavy atoms for all residues [d]	1.10 ± 0.07	0.90 ± 0.07	1.00 ± 0.11
Ramachandran statistics			
Most favored region (%)	89.6	90.2	92.0
Additionally allowed (%)	9.5	8.5	7.2
Generously allowed (%)	0.5	0.7	0.5
Disallowed (%)	0.5	0.7	0.3
WHAT_CHECK Z-scores			
1st generation packing quality	0.275	0.662	0.671
2nd generation packing quality	-0.883	-0.145	-0.667
Ramachandran plot appearance	-2.376	-2.836	-2.380
chi-1/chi-2 rotamer normality	-3.369	-3.504	-2.656
Backbone conformation	0.193	0.680	0.846
Inside/Outside distribution	1.060	0.965	0.956

[a] Residues for statistics include 3-9, 12-17, 23-28, 31-37, 47-61, 72-79, 90-108, 114-121, 123-132, 136-148, 154-159, 162-170.

[b] Residues for statistics include 3-8, 12-17, 22-28, 31-40, 45-60, 70-77, 88-105, 108-113, 116-125, 129-141, 147-152, 155-164.

[c] Residues for statistics include 3-9, 12-17, 23-28, 31-37, 47-61, 71-78, 89-110, 11 5-121, 123-131, 136-147, 154-159, 162-170.

[d] N- and C- terminal disordered residues are excluded.

Table S5.

Crystallographic data collection and refinement statistics.

Parameter	**RsgI2-PD**
PDB code	8HDJ
Crystallization	0.2 M Lithium sulfate monohydrate, pH 6.0, 20% w/v Polyethylene glycol 3350
Data collection[a]	
Space group	C 1 2 1
a,b,c (Å)	75.09, 101.53, 86.36
α, β, γ	90.00, 92.93, 90.00
Wavelength	0.979
Resolution (Å)	24.84-1.85 (1.89-1.85)
Unique reflections	54750 (3371)
Completeness（%）	99.4 (99.6)
Redundancy	3.1 (2.7)
Mean I/sigma (I)	9.3 (3.4)
R_{merge}[b]	0.070 (0.238)
Refinement	
R_{work}/R_{free}(%)	0.2292/0.2445
No. atoms	
Protein	4993
Water	481
B-factors	
Average B-factor	26.06
Proteins	24.81
Solvent	38.94
r.m.s.d.	
Bond length (Å)	0.006
Bond angles	1.05
Ramachandran statistics	
Favored (%)	99.36
Outliers (%)	0.00

a. Values in parentheses refer to data in the highest-resolution shell.

b. $R_{merge}=\Sigma_{hkl}\Sigma_i|I(hkl)_i-\langle I(hkl)\rangle|/\Sigma_{hkl}\Sigma_i\langle I(hkl)_i\rangle$, where I is the observed intensity, $\langle I(hkl)\rangle$ represents the average intensity, and $I(hkl)_i$ represents the observed intensity of each unique reflection.

Table S6.

Relative intensity of Xyn10Z and Xyn11B to CipA in SDS-PAGE gel, determined by the Quantity One Software.

Strain	Xyn10Z	Xyn11B
DSM1313	ND	ND
ΔrsgI6	1.93	0.28
ΔrsgI6::*rsgI6-T3*	1.86	0.26
ΔrsgI6::*rsgI6*	0.99	ND
ΔrsgI6::*rsgI6-T1*	0.44	ND
ΔrsgI6::*rsgI6-T2*	0.54	ND
ΔrsgI6::*rsgI6-T4*	0.39	ND
ΔrsgI6::*rsgI6-T5*	0.66	ND
ΔrsgI6Δrsep	2.03	0.26
ΔrsgI6Δrsep::*rsgI6-T3*	0.46	ND

Table S7.

Data values of Figs. 4D, 4E, 4F, and 5A.

Relative expression values of Fig. 4D

	Δ*rsgI6*	::*rsgI6*-T3	::*rsgI6*	::*rsgI6*-T1	::*rsgI6*-T2	::*rsgI6*-T4	::*rsgI6*-T5
sigI6	7.605	7.765	3.548	1.115	2.474	2.066	1.317
	14.390	8.676	2.261	0.998	2.341	1.392	2.579
	4.047	5.645	1.513	1.115	2.230	1.012	1.544
AVERAGE	8.681	7.362	2.441	1.076	2.348	1.490	1.813
STDEV	5.255	1.555	1.030	0.068	0.122	0.534	0.673
xyn10Z	10.273	26.368	1.694	4.230	20.262	8.698	6.638
	27.488	40.806	8.116	1.694	10.934	6.547	5.317
	15.356	23.600	4.143	2.950	12.560	3.607	9.387
AVERAGE	17.705	30.258	4.651	2.958	14.585	6.284	7.114
STDEV	8.845	9.239	3.241	1.268	4.983	2.556	2.076
xyn11B	377.598	508.713	14.327	30.289	75.098	46.874	56.914
	855.550	832.155	101.175	13.839	88.078	48.527	59.331
	337.960	576.313	66.289	24.263	99.093	31.357	45.277
AVERAGE	523.703	639.060	60.597	22.797	87.423	42.252	53.841
STDEV	288.071	170.606	43.703	8.322	12.011	9.472	7.514
xyn10D	26.085	121.528	5.408	19.095	38.993	47.675	33.478
	204.385	193.360	39.812	6.612	49.356	36.131	28.152
	66.494	132.069	20.045	14.076	58.694	18.317	34.900
AVERAGE	98.988	148.986	21.755	13.261	49.014	34.041	32.177
STDEV	93.486	38.789	17.266	6.281	9.855	14.790	3.557
cel9K	0.678	0.476	1.173	0.570	0.940	1.181	1.338
	1.173	0.763	0.664	1.548	0.683	1.338	0.993
	1.424	0.448	0.732	2.014	0.624	1.214	1.197
AVERAGE	1.092	0.562	0.856	1.377	0.749	1.244	1.176
STDEV	0.379	0.175	0.276	0.737	0.168	0.083	0.173

Relative expression values of Fig. 4E

	Δ*sigI6-rsgI6*	::*sigI6-rsgI6*	::*sigI6-rsgI6 P95A*	::*sigI6-rsgI6 N94A P95A*
sigI6	0.010	1.157	0.330	0.047
	0.028	1.057	0.188	0.054
	0.018	0.518	0.204	0.086
AVERAGE	0.019	0.910	0.241	0.062
STDEV	0.009	0.344	0.078	0.021
xyn10Z	0.631	1.725	0.479	0.354
	0.614	1.019	0.251	0.331

	0.649	0.677	0.485	0.280
AVERAGE	0.632	1.140	0.405	0.322
STDEV	0.018	0.535	0.133	0.038
xyn11B	0.056	0.620	0.102	0.084
	0.046	0.603	0.082	0.086
	0.030	0.376	0.140	0.062
AVERAGE	0.044	0.533	0.108	0.077
STDEV	0.013	0.136	0.029	0.013
xyn10D	0.761	4.459	1.941	0.394
	0.839	4.982	0.557	0.691
	0.456	2.229	1.786	0.645
AVERAGE	0.685	3.890	1.428	0.577
STDEV	0.203	1.462	0.758	0.160
cel9K	3.060	2.119	2.591	0.727
	3.418	2.664	3.715	0.525
	3.741	2.075	3.038	0.366
AVERAGE	3.406	2.286	3.115	0.539
STDEV	0.341	0.328	0.566	0.181

Relative xylanase values of Fig. 4F

	Δ*sigI6-rsgI6*	::*sigI6-rsgI6*	::*sigI6-rsgI6 P95A*	::*sigI6-rsgI6 N94A P95A*
	31.177	86.051	49.201	47.467
	16.818	94.565	34.972	52.237
	18.199	110.528	34.038	26.688
	27.232	84.352	49.322	
AVERAGE	23.356	93.874	41.883	42.131
STDEV	6.965	11.968	8.528	13.585

Relative expression values of Fig. 5A

	Δ*rsgI6* (1)	1::*rsgI6* -T3	Δ*rsgI6* Δ*rseP*(2)	2::*rsgI6* -T3
sigI6	7.605	7.765	2.842	0.597
	14.390	8.676	1.655	1.123
	4.047	5.645	2.634	1.170
AVERAGE	8.681	7.362	2.377	0.964
STDEV	5.255	1.555	0.634	0.318
xyn10Z	10.273	26.368	26.736	1.415
	27.488	40.806	11.638	2.190
	15.356	23.600	25.826	4.996
AVERAGE	17.705	30.258	21.400	2.867
STDEV	8.845	9.239	8.467	1.884
xyn11B	377.598	508.713	739.655	16.921

	855.550	832.155	257.907	35.278
	337.960	576.313	568.378	117.842
AVERAGE	523.703	639.060	521.980	56.680
STDEV	288.071	170.606	244.202	53.757
xyn10D	26.085	121.528	86.531	4.485
	204.385	193.360	50.743	10.162
	66.494	132.069	110.289	22.708
AVERAGE	98.988	148.986	82.521	12.452
STDEV	93.486	38.789	29.975	9.325
cel9K	0.678	0.476	2.645	1.341
	1.173	0.763	1.427	2.537
	1.424	0.448	1.721	1.782
AVERAGE	1.092	0.562	1.931	1.887
STDEV	0.379	0.175	0.635	0.605