Supplementary Information (SI) for Chemical Science. This journal is © The Royal Society of Chemistry 2024

Supplementary Information

Supplementary Figure	Figure Title	Page
1	Structural models of PcpA and SUMO-PcpX	5
2	Multiple sequence alignment of PcpY, PlpY and leader sequences of type II spliceotides	5
3	Multiple sequence alignment of PcpY, PlpY and leader sequences of type III spliceotides	5
4	Multiple sequence alignment of PcpY, PlpY and leader sequences of type V spliceotides	6
5	Structural models showing differences in the binding helix	6
6	LC-MS analysis for His_6 -PcpA cleaved with trypsin and GluC with different concentrations	7
	of SUMO-PcpY	
7	LC-MS analysis for His₀-PlpA3	8
8	LC-MS analysis for His ₆ -PlpA3-33	9
9	LC-MS analysis for His₀-PlpA3-45	10
10	LC-MS analysis for His6-PlpA3 and PlpXYfusion	11
11	LC-MS analysis for His6-PcpA and PcpXY _{fusion}	12
12	LC-MS analysis for His6-RhaA	13
13	LC-MS analysis for His ₆ -SUMO-RhaA _{core}	14
14	Localization of C ₈ H ₉ NO loss from His ₆ -SUMO-RhaA _{core}	15
15	LC-MS analysis for His6-mCherry109-RhaAcore	16
16	Localization of C ₈ H ₉ NO loss from His ₆ -mCherry109-RhaA _{core}	17
17	LC-MS analysis for His ₆ -SUMO-RhaA _{core} after 4.5 h, 24 h, and 48 h.	18
18	LC-MS analysis for His6-DHFR118-RhaAcore	19
19	LC-MS analysis for His6-MBP-Cterm-RhaAcore	20
20	LC-MS analysis for His ₆ -MBP154-RhaA _{core}	21
21	LC-MS analysis for DHFR-Nterm-RhaAcore-His6	22
22	LC-MS analysis for His ₆ -SUMO-Nterm-RhaA _{core}	23
23	Localization of C ₈ H ₉ NO loss from His ₆ -MBP-Cterm-RhaA _{core}	24
24	LC-MS analysis for His ₆ -SUMO-RhaA _{core} -N8	25
25	LC-MS analysis for His ₆ -SUMO-RhaA _{core} -N24	26
26	LC-MS analysis for His ₆ -SUMO-RhaA _{core} -N32	27
27	LC-MS analysis for His ₆ -SUMO-RhaA _{core} -N24C4	28
28	LC-MS analysis for His ₆ -SUMO-RhaA _{core} -N24C8	29
29	LC-MS analysis for His ₆ -SUMO-RhaA _{core} -N24C10	30

Methods

General

All reagents were purchased from commercial sources and used as received. Solvents were procured from Sigma-Aldrich and used as received. Double-distilled water was obtained from a PURELAB Chorus system (ELGA Veolia). Oligonucleotides for molecular cloning were synthesized by Microsynth (Switzerland). Plasmids were purified with the NucleoSpin plasmid purification kit purchased from Macherey-Nagel (Germany), agarose gel purifications were carried out with the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Germany). Q5® Site-Directed Mutagenesis kit, restriction enzymes, T4 DNA ligase and Q5 DNA polymerase were purchased from New England Biolabs (NEB, USA). Antibiotics (chloramphenicol, kanamycin, spectinomycin) were purchased from Applichem. GluC endoprotease was purchased from NEB (USA) and sequencing grade trypsin endoprotease was purchased from Promega. Bacteria lysis was done on a Qsonica Q700 sonicator equipped with either a 2 or 12 mm probe. Protino® agarose Ni-NTA resin was purchased from Macherey-Nagel (Germany). LC-MS experiments were performed on a Dionex Ultimate 3000 UHPLC equipped with columns from Phenomenex (USA) and coupled to a mass spectrometer. Mass spectra were acquired on an LTQ Orbitrap XL or Q Exactive (Thermo Fisher Scientific) spectrometer by using heated electrospray ionization (HESI). LC/MS data was analyzed with the Thermo Xcalibur Qual browser 4.1 (Thermo Fisher Scientific). Data analysis and statistical analysis were done in Microsoft Excel (2016) and Prism 9 (GraphPad).

Bioinformatic analysis

Multiple sequence alignments of PcpY, PlpY, and spliceotide leader sequences were made using MUSCLE in Geneious 7.1.9. Sequences were selected from previously reported precursor predictions,¹ and leader sequences were selected by truncating the sequences at the first double glycine motif in the sequence. Sequences not containing a double glycine motif were excluded from the analysis.

Structure predictions

Protein and protein complex structure predictions were conducted with AlphaFold Version 2.2.0 by providing FASTA files containing the amino acid sequences of the respective proteins.^{2,3} AlphaFold2 was accessed in the ETH Euler cluster through a bash script.

Plasmid construction

All plasmids used in this study are listed in Table S2. Protein production plasmids were constructed by Gibson Assembly® Cloning (for genes >100 base pairs) or Q5® site-directed mutagenesis (for genes <100 base pairs) protocol provided by New England Biolabs (USA). Genes encoding for His₆-SUMO-Rha_{core} truncation precursors were ordered from Twist Bioscience as gene fragments with flanking Ncol and BamHI restriction sites and subcloned into pACYCDuet vectors through restriction cloning (NEB). Gene assembly fragments were designed by NEBuilder tool online. Overlapping mutagenic primers were designed by NEBaseChanger tool online. A typical polymerase chain reaction (PCR) (50 µL) contained 20 ng template DNA, 1x Q5 reaction buffer, 200 µM dNTPs, 0.5 µM of each primer (Table S1), and 0.5 u Q5 High-Fidelity DNA Hot-Start Polymerase. The reaction was heated to 98 °C for 45 s followed by 35 cycles of 98 °C for 10 s, X °C for 20 s, and 72 °C for 20 s per kilobase DNA target sequence. The primer annealing temperature X was calculated with the NEB Tm calculator website or the NEBaseChanger (in case of the Q5 sitedirected mutagenesis protocol). The resulting PCR amplicons were treated with kinase-ligase-DpnI mix (KLD mix, NEB). Gibson Assembly® was performed with Gibson Assembly Master Mix (New England BioLabs) according to the manufacturer's instructions. DNA was visualized by 1% (w/v) agarose gel electrophoresis supplemented with ethidium bromide and GeneRuler™ 1kb DNA ladder (Thermo Scientific) marker. Plasmids were transformed into chemically competent E. coli DH5a strain (Invitrogen) and grown on LB agar containing appropriate antibiotics, then inoculated in corresponding liquid LB media. Plasmids were isolated from fresh overnight cultures and the gene-of-interest was sequence-verified by Microsynth AG (Switzerland). Sequence-verified plasmids were transformed into chemically competent *E. coli* BL21(DE3). All plasmids are under isopropyl-β-D-1-thiogalactopyranoside (IPTG) regulation. pACYCDuet encodes chloramphenicol resistance, pRSFDuet kanamycin resistance, and pCDFDuet spectinomycin resistance.

Protein expression and purification of substrate proteins

A Falcon tube containing 5 mL of LB medium was inoculated with *E. coli* BL21(DE3) cells taken from previously prepared glycerol stocks or from colonies on agar plates and supplemented with the appropriate antibiotics. The culture was shaken at 180 rpm overnight at 37 °C.

On the next day, 100 mL TB medium containing the appropriate antibiotics were inoculated with 1% v/v of this overnight culture and shaken at 37 °C until an OD₆₀₀ of 1.2 to 1.6 was reached, according to previously reported expressions in similar systems.⁴ After cooling the cultures at 4 °C for at least 20 min, 1 mM of IPTG was added and the cultures were

incubated on a shaker at 180 rpm at 16 °C for approximately 16 to 20 h. Subsequently, 40 mL from the cultures were centrifuged at 6000 × g for 10 min at 4 °C. The supernatant was discarded, and the cell pellets resuspended in 1 mL NPI-10 buffer. All NPI buffers were supplemented with 10% Glycerol. NPI buffers contain 50 mM NaH₂PO₄, 300 mM NaCl, 5 - 250 mM imidazole (where NPI-X contains X mM imidazole) and are adjusted to pH 8.0.

Cells resuspended in NPI-10 buffer were sonicated for 12 times 10 s, with 10 s of pause in between, at an amplitude of 30% to 40%. A sonication tip with a diameter of 2 mm was used, the resulting suspensions were kept on ice and centrifuged at 21000 x g for 30 min at 4 $^{\circ}$ C.

The supernatant was transferred to a new tube and 125 μ L of Protino[®] Ni-nitrilotriacetic acid (NTA) Agarose (Macherey-Nagel) was added. The samples were slowly shaken on a rotor at 10 rpm for at least 30 min. An appropriate polypropylene column was prewashed with NPI-10 buffer, the sample added, washed twice with NPI-10 (500 μ L), twice with NPI-20 (500 μ L), and the protein eluted with NPI-250 (550 μ L) and collected. Elution fractions were digested with appropriate endoproteinases. Protein splicing was analyzed by high-resolution LC-MS-MS. Samples were stored at -20 °C for further use.

Protein substrates for *in vitro* reactions were buffer-exchanged with PD MidiTrap G-25 columns (Cytiva) to reaction buffer (50 mM HEPES, 150 mM KCl, 10 % glycerol, pH 8) according to the manufacturer's protocol, frozen with liquid nitrogen and transferred to an anaerobic workstation (UNIIab pro, MBRAUN) for further use.

Time course of modification of His6-RhaA and His6-SUMO-Rhacore by RhaX and RhaXAleader

Protein expressions were set up as described above for His₆-RhaA and His₆-SUMO-Rhacore with either RhaX and RhaXA_{leader} in duplicates. After 4.5 h, 24 h, and 48 h a 10 mL aliquot of the expression cultures was taken and stored at -20 °C until purified as usual. Proteins were digested by trypsin and analyzed by LC-MS to quantify relative conversion and signals of modified and unmodified peptide.

Protein purification and reconstitution of radical SAM enzymes

A Falcon tube containing 10 mL of LB medium was inoculated with *E. coli* BL21(DE3) cells taken from previously prepared glycerol stocks or from single colonies on agar plates and supplemented with the appropriate antibiotics. The culture was shaken at 180 rpm overnight at 37 °C.

On the next day, two Ultrayield® 2.5 L flasks (Thomson) filled with 1 L TB medium containing the appropriate antibiotics were inoculated with 1% v/v of the overnight culture, supplemented with 25 μ M FeCl₃ and shaken at 37 °C until an OD₆₀₀ of around 1.5 was reached, according to previously reported expressions in similar systems.⁴ After cooling the cultures at 4 °C for at least 20 min, 1 mM of IPTG, 25 μ M FeCl₃, and 300 μ M L-Cys were added and the cultures were incubated on a shaker at 180 rpm at 16 °C for approximately 16 to 20 h. Subsequently, the cultures were centrifuged at 6000 × g for 10 min at 4 °C. The supernatant was discarded and the cell pellets resuspended in 4 mL NPI-5 buffer per gram cell pellet. Suspensions were supplemented with cOmplete Protease Inhibitor Cocktail (Roche), 2 mM DTT and 0.5 mg/mL lysozyme (Roth) and incubated at 4 °C for 1 h.

Cells resuspended in NPI-5 buffer were sonicated for 12 times 10 s, with 10 s of pause, at an amplitude of 50% to 70%. A sonication tip with a diameter of 12 mm was used. The resulting suspensions were kept on ice and centrifuged at $18000 \times g$ for 45 min at 4 °C.

The supernatant was transferred to a new tube and 4 mL of cOmplete His-tag purification resin (Roche) was added. The samples were slowly shaken on a rotor at 10 rpm for at least 30 min. Samples were centrifuged at 800 × g for 1 min, the supernatant carefully removed, and the resin resuspended in the same volume of NPI-5. This wash was repeated twice. An appropriate polypropylene column was prewashed with NPI-5 buffer, the sample added, washed twice with NPI-5 (5 column volumes), twice with NPI-20 (1 column volume), and the protein eluted with NPI-250 (three times 1 column volume) and pooled. Elution fractions were concentrated using 30 kDa MWCO Amicon-ULTRA-15 centrifugal filters to a volume of 0.5–1 mL. Samples were supplemented with 5 mM DTT, frozen with liquid nitrogen, and transferred to an anaerobic workstation.

After at least 16 h in an anaerobic environment, samples were buffer-exchanged using PD-10 columns (Cytiva) to reaction buffer (50 mM HEPES, 150 mM KCI, 10 % glycerol, pH 8) following the manufacturer's instructions. Samples were concentrated/diluted to around 250 µM and added with 20 equivalents DTT, 13 equivalents FeCl₂, 12 equivalents L-Cys and 1 µM of eclscS (*E. coli* cysteine desulfurase) and incubated overnight at 4 °C. Samples were then again buffer-exchanged to reaction buffer and concentrated to 250–1000 µM, frozen and stored for further use.

Analytical high-performance liquid chromatography-size exclusion chromatography

Reconstituted SUMO-tagged PcpX (57.7 kDa) or PcpXY (64.1 kDa) were incubated with PcpA (18.3 kDa) and transferred to air-tight vials prior to removal from the anaerobic chamber. Samples of individual components were similarly prepared for comparison, and all samples were separated by HPLC-SEC.

Anaerobic in vitro reactions

For anaerobic reactions, N-terminally His₆-tagged ecFldA and ecFpr from the ASKA collection⁵ were expressed according to the general protein expression and purification protocol. Elution fractions were concentrated, frozen and transferred to an anaerobic workstation and buffer-exchanged to reaction buffer with PD MidiTrap G-25 (Cytiva) columns.

Anaerobic reactions were carried out as follows: 50 µM splicease (SUMO-PcpX, SUMO-PcpXY or SUMO-PcpXY_{fusion}), 0–500 µM SUMO-PcpY, 100 µM PcpA, 20 µM ecFldA, 5 µM ecFpr, 2 mM NADPH, 10 mM DTT and filled with reaction buffer to 50 µL.⁶ Reactions were typically carried out at room temperature for 3 h, then removed from the glovebox and directly digested with appropriate proteases and analyzed by LC-MS-MS.

High-resolution mass spectrometry

Mass spectra were acquired on an LTQ Orbitrap XL, Q Exactive, or Ascend Tribrid (Thermo Fisher Scientific, USA) spectrometer by using heated electrospray ionization (HESI). The following method was used for analysis on LC-MS:

Solvent A: $H_2O + 0.1\%$ formic acid; solvent B: MeCN + 0.1% formic acid; column, Phenomenex Kinetex 2.6 µm C18-XB 100 Å (150 × 4.6 mm); flow rate, 0.7 mL/min; gradient: 95:5 A/B for 0.5 min ramped to 5:95 A/B over 20 min).

For MS-MS analysis, a normalized collision energy of 15 to 28 was used, depending on the observed fragmentation properties of peptide fragments. The MS instrument was operated in positive ionization mode at a scan range of 400–2000 *m/z* (or alternatively 700–1500 *m/z*), AGC target 2e5, maximum IT 100 ms and a resolution of 70000 at 400 *m/z*. The spray voltage was set to 5.0 kV, the probe heater temperature to 475 °C, and the capillary temperature to 270 °C. Columns were heated to 50 °C.

Supplementary Figures and Tables



Supplementary Figure S1. a) AlphaFold model of PcpA and SUMO-PcpX with the N-terminal MYG site (red) placed in the putative active site near the putative iron-binding cysteines (yellow). b) Structural model of PcpA and SUMO-PcpX with the two MYG sites (red and green) placed in the putative active site near the putative iron-binding cysteines (yellow).

	ļ	10	20	30	40	5 Q
pCDF-6xH-PcpY_translation	.MVENI	DNEREKSA	NEIEPESLLLP	RQAWQ <mark>S</mark> QIAYI	KAILKAKQAI	DRIE.KRYLR
pRSFDuet-1_PlpXYPlpY_translation	MNSNQI	I P N K V A T A A	QKSDDSSSVLP	RQGWQDKQAFI	IKALIKAKQS <mark>I</mark>	EIAEISNFLT
Raquimaris_B26				MKNVKLD1	[NHVVEAVEL <mark>I</mark>	NKVE
Rpleomorphica_PKS7				MMKNLQHDF	KNHIIEAAEL <mark>I</mark>	NKVE
Pphenolica_S4048				MKLSKQNF	KEVIKAEEL <mark>I</mark>	SQVE
PspARS97				MKLSKQNF	KEVIKAEEL <mark>I</mark>	JSQ <mark>VE</mark>
Prubra_W3				MKTIKQNF	KKLVIESQEL <mark>I</mark>	.TKIE
Pviridis_BBR56				MKTTKQNF	(KLVIESQEL <mark>I</mark>	.TKIE
Pluteoviolacea_2ta16				MKTSKQNF	KAVIQAEEL <mark>I</mark>	AKIE
Pluteoviolacea_MMG009				MKTSKQNF	KAVIQAEEL <mark>I</mark>	AKIE
Prubra_S2599				MKTTKQNV	/KAVIEAEEL <mark>I</mark>	AKIE
Prubra_\$2678				MKTTKQNV	/KAVIEAEEL <mark>I</mark>	AKIE
PspR3				MKTTKQNV	/KAVIEAEEL <mark>I</mark>	AKIE
Prubra_OCN096				MKTTKQNF	KAVIEAEEL <mark>I</mark>	AKIE
PsrA				MKTTKQNF	(KAVIEAEQL <mark>I</mark>	AKIE
PphA				MKNVHQDE	FHDLSEAKDL	QQVE
Psp2A				MKDLHQNN	1QDVTEAKEL <mark>I</mark>	VKVE
Cbacterium_ARS1043				MKQTSKDM	4 K E L T K A K A L <mark>I</mark>	ESIE
Pphenolica_S4048				MRIKNYKM	INELNEAAEL <mark>I</mark>	AQVE
PsplA				MRNKNYDM	10 E L NN A A E L	ELVD

Supplementary Figure S2. Multiple sequence alignment of PcpY, PlpY, and leader sequences of type II spliceotides.¹ R._aquimaris_B26 is the RhaA leader peptide. Sequences were aligned with MUSCLE in Geneious 7.1.9 and the alignment image was generated using ESPript 3.0.⁷

	1	10	20	30	40	50
pCDF-6xH-PcpY_translation	.MVEN]	IDNEREKSAN	JE <mark>IEPESLL<mark>P</mark>I</mark>	QAWQSQIAY	LKAILKAKQA	LDRIEKRYLR.
pRSFDuet-1_PlpXYPlpY_translation	MNSNQJ	ΙΡΝΚΥΑΤΑΑς	QKSDDSSSVL <mark>P</mark> I	R <mark>Q</mark> GWQDKQAE	FIKALIKAKQS	LEIAEISNFLT
ThnA	MN]	INGSNSS	<mark>L</mark> F <mark>P</mark> I	QPQGDQSS.	AVRRAN	NQIRQSC
Proteobacteria_bacterium_DOLZORAL124_45_7	MD	VP <mark>N</mark> NNR	V L <mark>P I</mark>	RTPDTQIQT.	QNASDT	LNQYAAMNGR.
Teikelboomii_ATCC_49788	MD1	IQTRRGQNT.	I L <mark>P</mark> I	R <mark>Q</mark> PDTQSQT.	VQARQR	LDQFNGCNPNL
TspBin_8_2_c_00000059211	MD]	IQTRRGQNT.	I L <mark>P</mark> I	RQPNTQNQT.	LQARQR	LDQFNGCSPSL
Tcaldifontis_DSM_21228	MD]	IQSRNKANTS	SPVLP	RS		
Tfructosivorans_ATCC_49748	<u>M</u> D]	IQNRNKANTS	5 P V L P	<u>RS</u>	• • • • • • • • • • •	

Supplementary Figure S3. Multiple sequence alignment of PcpY, PlpY, and leader sequences of type III spliceotides.¹ Sequences were aligned with MUSCLE in Geneious 7.1.9 and the alignment image was generated using ESPript 3.0.⁷

	ŗ	10	20	зo	40	5 <u>0</u>
pCDF-6xH-PcpY_translation	.MVEN	IDNEREKSAN	EIEPESLLLP	ROAWQSQIAY	LKAILKAKQA	LDRIEK. RYLR.
pRSFDuet-1_PlpXYPlpY_translation	MNSNQ	I P N K V A T A A Q	KSDDSSSVLP	ROGWODKOAF	IKALIKAKQS	SLEIAEIS.NFLT.
Cferrugineus_Cbfe23	. MTT <mark>K</mark>	T	PRNDVLD	RQS	ERTRKV	/LELDEKNLENVT.
CyiA	. MTT <mark>K</mark>	P E K K F	PRTEILD	RLS	ERTRK	/LELEEKNLENVT.
Cfuscus_DSM_2262	.MTT <mark>K</mark>	T E K K F	PRTEILN	I <mark>R</mark> QS	ERTRKV	/LELEEKNLENVT.
Aviolaceum_SDU8	MNKTE	ΚΚΡV F	PRTEVVE	R <mark>Q</mark> K	ERARKI	LDLDKESLETVR.
Cfuscus_DSM_2262	. MTT <mark>K</mark>	VEKKL	PRTQVIE	8 <mark>R</mark> QK	ERTREN	/LEIEEGSLDTVR.
CyrA	. MTT <mark>K</mark>	VEKKL	PRTQVIE	2 <mark>R</mark> QK	ERTREN	/LEIEEGSLDTVR.
Aviolaceum_Cb_vi76	.MMK <mark>K</mark>	S <mark>EKKF</mark>	PRTQVIE	R <mark>Q</mark> N	ERALQV	/LELDEKALDQIR.
AreA	. MTE <mark>K</mark>	I E K K F	PRTQVIE	R <mark>Q</mark> N	ERALQV	/LELDETALEQIR.
ArdA	. MTK <mark>K</mark>	VEKKF	PRTQVIE	R <mark>Q</mark> N	ERALQV	/LELDEKALEQIR.
Agephyra_DSM_2261	. MTK <mark>K</mark>	VEKKF	PRTQVIE	RQN	ERTRQ	/LELDEKALDQIR.
Aviolaceum_Cb_vi76	MK <mark>K</mark>	VEKKF	PRTQVIE	R <mark>Q</mark> N	ERTRQV	/LELDEKALEQIR.
Aprimigenium_ATCC_29037	MT <mark>K</mark>	I	PRTEVIE	RQE	EVARD	LELDENTLEGVR.
Agephyra_DSM_2261	. MTT <mark>K</mark>	VEKKF	PRSKVIE	R _Q N	EKAREV	VELDENTLEGVR.
Mboletus_DSM_14713	. MTT <mark>K</mark>	VEKKF	PRSKVIE	R <mark>Q</mark> N	ERAREN	/LELDENSLESVRG
ArkA	M T <mark>K</mark>	VEKKF	PRSKVIE	R <mark>Q</mark> N	EKAREV	/LELDEGSLESVR.
Aviolaceum_Cb_vi76	. MMT <mark>K</mark>	V E K K F	PRSNVIE	RQN	EKAREV	/LELDEGTLESVR.
Aviolaceum_Cb_vi76	MT <mark>K</mark>	VEKKF	PRSNVIE	RQN	EKARE	LELDEGTLESVR.

Supplementary Figure S4. Multiple alignment of PcpY, PlpY, and leader sequences of type V spliceotides.¹ Sequences were aligned with MUSCLE in Geneious 7.1.9 and the alignment image was generated using ESPript 3.0.⁷



Supplementary Figure S5. a) AlphaFold model of PIpA3 and PIpX in the presence (top) and absence (bottom) of PIpY. Without PIpY, the binding helix is not replaced by PIpA3. **a**) AlphaFold model of PcpA and PcpX in the presence (top) and absence (bottom) of PcpY. Without PcpY, the binding helix is not replaced by PcpA.



Supplementary Figure S6. LC-MS analysis for His₆-PcpA cleaved with trypsin and GluC to give the peptide fragment LVTAVGGVTGGSGIYGPIQAMYGAVVGDPKPGK. Extracted ion chromatograms for m/z 1039.5531 (precursor, [M+3H]³⁺), m/z 994.5303 (modified, [M+3H]³⁺) for *in vitro* enzymatic reactions of His₆-PcpA and SUMO-PcpX with 0 (**a**), 0.1 (**b**), 0.5 (**c**), 1 (**d**), 2 (**e**), 10 (**f**), or 40 (**g**) equivalents SUMO-PcpY. Extracted mass spectra for *in vitro* enzymatic reactions of His₆-PcpA and SUMO-PcpX with 0 (**b**), 0.5 (**c**), 1 (**d**), 2 (**e**), 10 (**h**), 0.1 (**j**), 0.5 (**k**), 1 (**l**), 2 (**m**), 10 (**n**), or 40 (**o**) equivalents SUMO-PcpY. "Modified" refers to excision of tyramine (-C₆H₉NO).



Supplementary Figure S7. LC-MS analysis for His_6 -PlpA3 cleaved with trypsin to give the peptide fragment AVAAMYGVVFPWDNEFPWPR. Extracted ion chromatograms for m/z 1176.5671 (precursor, $[M+2H]^{2+}$), m/z 1109.0329 (modified, $[M+2H]^{2+}$) for **a**) precursor only expression, **b**) precursor + PlpX co-expression, and **c**) precursor + PlpXY co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + PlpX co-expression, and **f**) precursor + PlpXY co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S8. LC-MS analysis for His₆-PlpA3-33 cleaved with trypsin to give the peptide fragment AVAAMYGVVFPWDNEFPWPR. Extracted ion chromatograms for m/z 1176.5671 (precursor, $[M+2H]^{2+}$), m/z 1109.0329 (modified, $[M+2H]^{2+}$) for **a**) precursor only expression, **b**) precursor + PlpX co-expression, and **c**) precursor + PlpXY co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + PlpX co-expression, and **f**) precursor + PlpXY co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S9. LC-MS analysis for His₆-PlpA3-45 cleaved with trypsin to give the peptide fragment AVAAMYGVVFPWDNEFPWPR. Extracted ion chromatograms for m/z 1176.5671 (precursor, $[M+2H]^{2+}$), m/z 1109.0329 (modified, $[M+2H]^{2+}$) for **a**) precursor only expression, **b**) precursor + PlpX co-expression, and **c**) precursor + PlpXY co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + PlpX co-expression, and **f**) precursor + PlpXY co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S10. LC-MS analysis for His₆-PlpA3 cleaved with trypsin to give the peptide fragment AVAAMYGVVFPWDNEFPWPR. Extracted ion chromatograms for m/z 1176.5671 (precursor, [M+2H]²⁺), m/z 1109.0329 (modified, [M+2H]²⁺) for **a**) precursor only expression, **b**) precursor + PlpX co-expression, **c**) precursor + PlpXY co-expression, and **d**) precursor + PlpXY_{fusion} co-expression. Extracted mass spectra for **e**) precursor only expression, **f**) precursor + PlpX co-expression, **g**) precursor + PlpXY co-expression, **and h**) precursor + PlpXY_{fusion} co-expression, and **h**) precursor + PlpXY_{fusion} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S11. LC-MS analysis for His₆-PcpA cleaved with trypsin and GluC to give the peptide fragment LVTAVGGVTGGSGIYGPIQAMYGAVVGDPKPGK (**a**, **c**, **e**, **h**, **j**, **l**, **n**, **p**) or FPSPLPKPSPIPSPWKPPVDVQPMYGVVSNDS (**b**, **d**, **f**, **h**, **k**, **m**, **o**, **q**). Extracted ion chromatograms for: *m*/*z* 1039.5531 (precursor, [M+3H]³⁺), *m*/*z* 994.5303 (modified, [M+3H]³⁺), (**a**, **c**, **e**, **g**, **j**, **l**, **n**, **p**) ; or *m*/*z* 1186.9496 (precursor, [M+3H]³⁺), *m*/*z* 1141.9267.5303 (modified, [M+3H]³⁺) (**b**, **d**, **f**, **h**, **k**, **m**, **o**, **q**) for *in vitro* enzymatic reactions of His₆-PcpA and SUMO-PcpX (**a**, **b**), SUMO-PcpX + 10 eq. SUMO-PcpY (**c**, **d**), SUMO-PcpX (**e**, **f**), or PcpXY_{fusion} (**g**, **h**). Extracted mass spectra for *in vitro* enzymatic reactions of His₆-PcpA and SUMO-PcpX (**j**, **k**), SUMO-PcpX + 10 eq. SUMO-PcpY (**l**, **m**), SUMO-PcpXY (**n**, **o**), or PcpXY_{fusion} (**p**, **q**). "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S12. LC-MS analysis for His₆-RhaA cleaved with trypsin to give the peptide fragment IDSSVFTHCDFFVCGVALYGSVIDPHRFDMLP. Extracted ion chromatograms for m/z 1196.5647 (precursor, [M+3H]³⁺), m/z 1151.5419 (modified, [M+3H]³⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaX co-expression, **f**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₆H₉NO).



Supplementary Figure S13. LC-MS analysis for His_6 -SUMO-RhaA_{core} cleaved with trypsin to give the peptide fragment IDSSVFTHCDFFVCGVALYGSVIDPHRFDMLP. Extracted ion chromatograms for m/z 1196.5647 (precursor, [M+3H]³⁺), m/z 1151.5419 (modified, [M+3H]³⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaX co-expression, **f**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₆H₉NO).



Supplementary Figure S14. Localization of C₈H₉NO loss from His₆-SUMO-RhaA_{core} cleaved with trypsin to give the peptide fragment IDSSVFTHCDFFVCGVALYGSVIDPHRFDMLP. a) Summary of observed b (above, purple) and y (below, orange) fragmentation ions. b) MS/MS spectrum from parallel reaction monitoring (PRM)-mediated fragmentation (CE 22) from m/z 1151.2139 ([M+3H]³⁺) parent ion. Observed b and y ions are indicated as before.



Supplementary Figure S15. LC-MS analysis for His₆-mCherry209-RhaA_{core} cleaved with trypsin to give the peptide fragment IDSSVFTHCDFFVCGVALYGSVIDPHRFDMLPGGAYNVNIK. Extracted ion chromatograms for *m*/*z* 1126.7945 (precursor, [M+4H]⁴⁺), *m*/*z* 1093.0274 (modified, [M+4H]⁴⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaX co-expression, **f**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S16. Localization of C₈H₉NO loss from His₆-mCherry209-RhaA_{core} cleaved with trypsin to give the peptide fragment DSSVFTHCDFFVCGVALYGSVIDPHRFDMLPGGAYNVNIK. **a**) Summary of observed b (above, purple) and y (below, orange) fragmentation ions. **b**) MS/MS spectrum from parallel reaction monitoring (PRM)-mediated fragmentation (CE 22) from *m*/z 874.6296 ([M+5H]⁵⁺) parent ion. Observed b and y ions are indicated as before.



Supplementary Figure S17. LC-MS based conversion (**a**), MS intensities (logarithmic scale) of unmodified (**b**, *m/z* 1196.5647 [M+3H]³⁺), and modified (**c**, *m/z* 1151.5419 [M+3H]³⁺) tryptic peptide fragments of expressions of His₆-RhaA or His₆-SUMO-RhaAcore with RhaX or RhaXA_{leader} purified at 4.5 h, 24 h, and 48 h after induction



Supplementary Figure S18. LC-MS analysis for His₆-DHFR118-RhaA_{core} cleaved with trypsin to give the peptide fragment IDSSVFTHCDFFVCGVALYG SVIDPHRFDMLPGDTHYPDYEPDDWER. **a**) Extracted ion chromatograms for m/z 1093.4884 (precursor, [M+5H]⁵⁺), m/z 1066.4748 (modified, [M+5H]⁵⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S19. LC-MS analysis for His₆-MBP-Cterm-RhaA_{core} cleaved with trypsin to give the peptide fragment IDSSVFTHCDFFVCGVALYGSVIDPHRFDMLP. **a**) Extracted ion chromatograms for m/z 1196.5647 (precursor, [M+3H]³⁺), m/z 1151.5419 (modified, [M+3H]³⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaX co-expression, **f**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S20. LC-MS analysis for His₆-MBP154-RhaA_{core} cleaved with trypsin to give the peptide fragment IDSSVFTHCDFFVCGVALYGSVIDPHRFDMLPALDKSVIDPHRFDMLP. **a**) Extracted ion chromatograms for m/z 1004.4861 (precursor, [M+4H]⁴⁺), m/z 970.7190 (modified, [M+4H]⁴⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaX co-expression, **f**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S21. LC-MS analysis for DHFR-Nterm-RhaA_{core}-His₆ cleaved with AspN to give the peptide fragment DFFVCGVALYGSVI. **a**) Extracted ion chromatograms for m/z 775.3734 (precursor, [M+2H]²⁺), m/z 677.8392 (modified, [M+2H]²⁺) for precursor + RhaXA_{leader} co-expression. **b**) Extracted mass spectra precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S22. LC-MS analysis for His₆-SUMO-Nterm-RhaA_{core} cleaved with trypsin to give the peptide fragment IDSSVFTHCDFFVCGVALYG SVIDPHRFDMLPLVPR. **a**) Extracted ion chromatograms for m/z 1014.0019 (precursor, [M+4H]⁴⁺), m/z 980.2348 (modified, [M+4H]⁴⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaX co-expression, **f**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S23. Localization of C8H9NO loss from His6-MBP-Cterm-RhaAcore cleaved with AspN to give the peptide fragment DFFVCGVALYGSVI. a) Summary of observed b (above, purple) and y (below, orange) fragmentation ions. b) MS/MS spectrum from parallel reaction monitoring (PRM)-mediated fragmentation (CE 22) from *m/z* 677.8410 ([M+2H]²⁺) parent ion. Observed b and y ions are indicated as before.



Supplementary Figure S24. LC-MS analysis for His₆-SUMO-RhaA_{core}-N8 cleaved with trypsin to give the peptide fragment IDSSVFTHCDFFVCGVALYGSVIDPHRFDMLP. Extracted ion chromatograms for m/z 1196.5647 (precursor, [M+3H]³⁺), m/z 1151.5419 (modified, [M+3H]³⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaX co-expression, **f**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S25. LC-MS analysis for His₆-SUMO-RhaA_{core}-N24 cleaved with trypsin to give the peptide fragment AEQTPDELEMEDGDEIDAMLHQTGGHHCDFFVCGVALYGSVIDPHRFDMLP. Extracted ion chromatograms for m/z 1422.6294 (precursor, [M+4H]⁴⁺), m/z 1388.8622 (modified, [M+4H]⁴⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S26. LC-MS analysis for His₆-SUMO-RhaA_{core}-N32 cleaved with trypsin to give the peptide fragment AEQTPDELEMEDGDEIDAMLHQTGGHVALYGSVIDPHRFDMLP. Extracted ion chromatograms for m/z 1195.5466 (precursor, [M+4H]⁴⁺), m/z 1161.7795 (modified, [M+4H]⁴⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaX co-expression, **f**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S27. LC-MS analysis for His₆-SUMO-RhaA_{core}-N24-C4 cleaved with trypsin to give the peptide fragment AEQTPDELEMEDGDEIDAMLHQTGGHHCDFFVCGVALYGSVIDPHRF. Extracted ion chromatograms for *m*/*z* 1047.0641 (precursor, $[M+5H]^{5+}$), *m*/*z* 1020.0504 (modified, $[M+5H]^{5+}$) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaX co-expression, **f**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S28. LC-MS analysis for His₆-SUMO-RhaA_{core}-N24-C8 cleaved with trypsin to give the peptide fragment AEQTPDELEMEDGDEIDAMLHQTGGHHCDFFVCGVALYGSVID. Extracted ion chromatograms for m/z 1174.2580 (precursor, [M+4H]⁴⁺), m/z 1140.4909 (modified, [M+4H]⁴⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaX co-expression, **f**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).



Supplementary Figure S29. LC-MS analysis for His₆-SUMO-RhaA_{core}-N24-C10 cleaved with trypsin to give the peptide fragment AEQTPDELEMEDGDEIDAMLHQTGGHHCDFFVCGVALYGSV. Extracted ion chromatograms for m/z 1117.2302 (precursor, [M+4H]⁴⁺), m/z 1083.4631 (modified, [M+4H]⁴⁺) for **a**) precursor only expression, **b**) precursor + RhaX co-expression, **c**) precursor + RhaXA_{leader} co-expression. Extracted mass spectra for **d**) precursor only expression, **e**) precursor + RhaX co-expression, **f**) precursor + RhaXA_{leader} co-expression. "Modified" refers to excision of tyramine (-C₈H₉NO).

Table St. I millers used in time study	Table S1	. Primers	used in	this	study
--	----------	-----------	---------	------	-------

Name	Sequence (5'-3')
PlpY-PlpA3-N33_plpA3_fwd	TTATCTAAAGAAGAACGCCAACAAC
PlpY-PlpA3-N33_plpA3_rev	GCCGCTGCTGTGATGATG
PlpY-PlpA3-N33_plpy_fwd	ATCATCATCACAGCAGCGGCAACTCTAATCAAATACCAAATAAAG
PlpY-PlpA3-N33_plpy_rev	TGGCGTTCTTCTTTAGATAATGTCAGAAAATTGCTAATTTC
PlpY-PlpA3-N45_plpA3_fwd	TCTGGCTATGATTTCACTGCC
PlpY-PlpA3-N45_plpA3_rev	GCCGCTGCTGTGATGATG
PlpY-PlpA3-N45_plpy_fwd	ATCATCACAGCAGCGGCAACTCTAATCAAATACCAAATAAAG
PlpY-PlpA3-N45_plpy_rev	GCAGTGAAATCATAGCCAGATGTCAGAAAATTGCTAATTTC
PcpXYfusion_fwd	ATGGTCGAAAATATAGACAAC
PcpXYfusion _rev	ATCAACTACAGCACCAATC
RhaXY_Cfusion_fwd	GTTGAGGCTGTAGAGCTACTAAACAAAGTTGAATAGCTCGAGTCTGGTAAAG
RhaXY_Cfusion_rev	AACATGGTTTGTATCAAGTTTTACGTTTTCATGCTGGCTATTGCGATACC
PlpXYfusion_PlpX_fwd	TAACTCGAGTCTGGTAAAG
PlpXYfusion_PlpX_rev	CTTTGCTAAAGCGTAAGC
PlpXYfusion_PlpY_fwd	CTGCTTACGCTTTAGCAAAGATGAACTCTAATCAAATACCAAATAAAG
PlpXYfusion_PlpY_rev	TCTTTACCAGACTCGAGTTATTATGTCAGAAAATTGCTAATTTC
mCherry_Rha_fwd	CTCCTCGGTTTTTACCCACTGTGACTTTTTCGTTTGTGGTGTAGCGCTGTACGGCAGC
mCherry_Rha_rev	TCGATACGCTGACCGGTAGCGTTAATGCCGCCGCCGGGCAGCTG
pACYC_Rha_fwd	GGCGGGATTAACGCTACC
pACYC_Rha_rev	AGGCAACATATCGAAACGATG
MBP_Cterm_fwd	ATCGTTTCGATATGTTGCCTTAATCGTATTGTACACGGC
MBP_Cterm_rev	CCGGTAGCGTTAATCCCGCCCTTTCTGTTCGACTTAAGC
MBP_154_fwd	ATCGTTTCGATATGTTGCCTGCGCTGGATAAAGAACTGAAAG
MBP_154_rev	CCGGTAGCGTTAATCCCGCCCGGGATCTCTTCCCAGGTTTTTG
SUMO_nterm_fwd	ATCGTTTCGATATGTTGCCTTTAGTTCCTCGTGGTTCAG
SUMO_nterm_rev	CCGGTAGCGTTAATCCCGCCACCGCTGCTATGATGATG
DHFR_nterm_fwd	ATCGTTTCGATATGTTGCCTAGCCAGGATCCGGAGAATG
DHFR_nterm_rev	CCGGTAGCGTTAATCCCGCCCATGGTATATCTCCTTATTAAAGTTAAACAAAATTATTTC
DHFR_118_fwd	ATCGTTTCGATATGTTGCCTGGGGATACCCATTATCCG
DHFR_118_rev	CCGGTAGCGTTAATCCCGCCTTCTACTTCGGCGTCAATG

Table S2. Plasmids used in this study.

Plasmid	Description
<i>plpA3</i> /pACYCDuet-1	PlpA3 precursor protein with an N-terminal His_6 -tag and a Factor Xa cleavage site under IPTG regulation and with chloramphenicol resistance (from here on referred to as PlpA3 (Morinaka, 2018))
<i>plpXY</i> /pRSFDuet	Splicease PlpX together with its accessory protein PlpY under IPTG regulation and with kanamycin resistance (referred to as PlpXY) (Morinaka, 2018)
sumo-pcpY/pCDF	Accessory protein PcpY with an N-terminal His $_6$ -tag, SUMO-tagged under IPTG regulation and with spectinomycin resistance (referred to as SUMO-PcpY)
sumo-pcpX/pCDF	Splicease PcpX with an N-terminal His_6 -tag, SUMO-tagged under IPTG regulation and with spectinomycin resistance (referred to as SUMO-PcpY)
sumo-pcpXY/pCDF	Splicease PcpX with an N-terminal His ₆ -tag and the accessory protein PcpY, Pcpx is SUMO-tagged under IPTG regulation and with spectinomycin resistance (referred to as SUMO-PcpY)
<i>рсрА</i> /pCDF	PcpA precursor protein with an N-terminal His6-tag, under IPTG regulation and with spectinomycin resistance (referred to as PcpA (Morinaka, 2018))
<i>plpX</i> /pRSFDuet	Splicease PIpX under IPTG regulation and with kanamycin resistance (referred to as PIpXY)
<i>plpA3-33</i> /pACYCDuet-1	PIpA3 precursor protein with an N-terminal His ₆ -tag and a Factor Xa cleavage site, the first 33 amino acids replaced by PIpY, under IPTG regulation and with chloramphenicol resistance (from here on referred to as PIpA3-33)
plpA3-45/pACYCDuet-1	PIpA3 precursor protein with an N-terminal His ₆ -tag and a Factor Xa cleavage site, the first 45 amino acids replaced by PIpY, under IPTG regulation and with chloramphenicol resistance (from here on referred to as PIpA3-45)
plpXYfusion/pRSFDuet	Splicease PIpX fused to its accessory protein PIpY under IPTG regulation and with kanamycin resistance (referred to as PIpXYfusion)
rhaA/pACYC	RhaA precursor protein with an N-terminal His ₆ -tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as RhaA)
sumo-rhaA/pACYC	SUMO with C-terminal RhaA core and N-terminal His6-tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as SUMO-RhaAcore)
sumo-nterm-rhaA/pACYC	SUMO with N-terminal RhaA core and N-terminal His ₆ -tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as RhaAcore-SUMO)
mbp-rhaA/pACYC	MBP with C-terminal RhaA core and N-terminal His ₆ -tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as MBP-RhaAcore)
mbp-154-rhaA/pACYC	MBP with internal RhaA core and N-terminal His6-tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as MBP-154-RhaAcore)
dhfr-118-rhaA/pACYC	DHFR with internal RhaA core and N-terminal His ₆ -tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as DHFR-118-RhaAcore)
mCherry-209-rhaA/pACYC	mCherry with internal RhaA core and N-terminal His6-tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as mCherry-209-RhaAcore)

rhaX/pRSF	Splicease RhaX under IPTG regulation and with kanamycin resistance (referred to as RhaX)
rhaXAleader/pRSF	Splicease RhaX with C-terminal fusion of the RhaA leader under IPTG regulation and with kanamycin resistance (referred to as $RhaXA_{leader}$)
sumo-rhaA_N8del/pACYC	SUMO with C-terminal RhaA core and N-terminal His ₆ -tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as SUMO-RhaAcore). Truncation of the cleavage site and core by 8 N-terminal amino acids.
sumo-rhaA_N24del/pACYC	SUMO with C-terminal RhaA core and N-terminal His ₆ -tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as SUMO-RhaAcore). Truncation of the cleavage site and core by 24 N-terminal amino acids.
sumo-rhaA_N32del/pACYC	SUMO with C-terminal RhaA core and N-terminal His ₆ -tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as SUMO-RhaAcore). Truncation of the cleavage site and core by 32 N-terminal amino acids.
sumo-rhaA_N24_C4del/pACYC	SUMO with C-terminal RhaA core and N-terminal His ₆ -tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as SUMO-RhaAcore). Truncation of the cleavage site and core by 24 N-terminal and 4 C-terminal amino acids.
sumo-rhaA_N24_C8del/pACYC	SUMO with C-terminal RhaA core and N-terminal His ₆ -tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as SUMO-RhaAcore). Truncation of the cleavage site and core by 24 N-terminal and 8 C-terminal amino acids.
sumo-rhaA_N24_C10del/pACYC	SUMO with C-terminal RhaA core and N-terminal His ₆ -tag under IPTG regulation and with chloramphenicol resistance (from here on referred to as SUMO-RhaAcore). Truncation of the cleavage site and core by 24 N-terminal and 10 C-terminal amino acids.

 Table S3. Protein sequences used in this study. The precursor (minimal) core sequence is underlined. Splicease modification sites are shown in bold.

Protein	Sequence
His ₆ -PlpA3	GSSHHHHHHSSGLVPRGSHMSIESAKAFYQRMTDDASFRTPFEAELSKEERQQLIKDSGYDFTAEEW
	QQAMTEIQAARSNEELNEEELEAIAGR <u>AVAAMYGVVFPWDNEFPWPRWGG</u>
His ₆ -PlpYA3-33	MGSSHHHHHHSSGNSNQIPNKVATAAQKSDDSSSVLPRQGWQDKQAFIKALIKAKQSLEIAEISNFLTL
	SKEERQQLIKDSGYDFTAEEWQQAMTEIQAARSNEELNEEELEAIDGR <u>AVAAMYGVVFPWDNEFPWP</u>
	RWGG
His ₆ -PlpYA3-45	MGSSHHHHHHSSGNSNQIPNKVATAAQKSDDSSSVLPRQGWQDKQAFIKALIKAKQSLEIAEISNFLT
	SGYDFTAEEWQQAMTEIQAARSNEELNEEELEAIDGR <u>AVAAMYGVVFPWDNEFPWPRWGG</u>
His ₆ -PcpA	MGSSHHHHHHSSGENLYFQSHMSSNILEKVKEFFVRLVKDDAFQSQLQNNSIDEVRNILQEAGYIFSK
	EEFETATIELLDLKERDEFHELTEEELVTAVGG <u>VTGGSGIYGPIQAMYGAVVGDPKPGKDWGWRFPSP</u>
	LPKPSPIPSPWKPPVDVQPMYGVVVSNDS
His ₆ -SUMO-PcpX	MGSHHHHHHHSSGLVPRGSASHINLKVKGQDGNEVFFRIKRSTQLKKLMNAYCDRQSVDMTAIAFLF
	DGRRLRAEQTPDELEMEDGDEIDAMLHQTGGHMTYRRTSYAVWEITLKCNLACSHCGSRAGHTRAK
	ELSTQEALDLVRQMADVGIIEVTLIGGEAFLRPDWLQIAEAITKAGMLCSMTTGGYGISLETARKMKAA
	GIASVSVSIDGLEETHDRLRGRKGSWQAAFKTMSHLREVGIFFGCNTQINRLSAPEFPLIYERIRDAGA
	RAWQIQLTVPMGRAADNANILLQPYELLDLYPMIARVARRARQEGVQIQPGNNIGYYGPYERLLRGRG
	SDSEWAFWQGCAAGLSTLGIEADGAIKGCPSLPTSAYTGGNIREHSLREIVEESEQLRFNLGAGTSQG
	TAHLWGFCQTCEFSELCRGGCTWTAHVFFNRRGNNPYCHHRALFQAEQGIRERVVPKVEAQGLPFD
	NGEFELIEEPIDAPLPENDPLHFTSDLVQWSASWQEESESIGAVVD
His ₆ -SUMO-PcpY	MGSHHHHHHHSSGLVPRGSASHINLKVKGQDGNEVFFRIKRSTQLKKLMNAYCDRQSVDMTAIAFLF
	DGRRLRAEQTPDELEMEDGDEIDAMLHQTGGHMVENIDNEREKSANEIEPESLLLPRQAWQSQIAYLK
	AILKAKQALDRIEKRYLR
PcpY	MVENIDNEREKSANEIEPESLLLPRQAWQSQIAYLKAILKAKQALDRIEKRYLR

His₀-SUMO-	MGSHHHHHHHSSGLVPRGSASHINLKVKGQDGNEVFFRIKRSTQLKKLMNAYCDRQSVDMTAIAFLF
PcpXYfusion	DGRRLRAEQTPDELEMEDGDEIDAMLHQTGGHMTYRRTSYAVWEITLKCNLACSHCGSRAGHTRAK
	ELSTQEALDLVRQMADVGIIEVTLIGGEAFLRPDWLQIAEAITKAGMLCSMTTGGYGISLETARKMKAA
	GIASVSVSIDGLEETHDRLRGRKGSWQAAFKTMSHLREVGIFFGCNTQINRLSAPEFPLIYERIRDAGA
	RAWQIQLTVPMGRAADNANILLQPYELLDLYPMIARVARRARQEGVQIQPGNNIGYYGPYERLLRGRG
	SDSEWAFWQGCAAGLSTLGIEADGAIKGCPSLPTSAYTGGNIREHSLREIVEESEQLRFNLGAGTSQG
	TAHLWGFCQTCEFSELCRGGCTWTAHVFFNRRGNNPYCHHRALFQAEQGIRERVVPKVEAQGLPFD
	NGEFELIEEPIDAPLPENDPLHFTSDLVQWSASWQEESESIGAVVDMVENIDNEREKSANEIEPESLLL
	PRQAWQSQIAYLKAILKAKQALDRIEKRYLR
PlnX	MTKKYRRVSYAVWEITI KCNI ACSHCGSRAGOARTKEI STEEAENI VROLADVGIKEVTI IGGEAEMR
1 ip/(SDWI FLAKAVTEAGMICGMTTGGEGVSI ETABKMKEAGIKTVSVSIDGGIDETHDPODGKKGAWHSAE
	DTMSLIARAVI LAGMILGUITIGGI GVSLL TARMINELAGIRT VSVIDGGIFT I TDRQRGRRGRWIJSAI
	TAAY I GGNIKDKPLREIVEQ I EELK FNLKAG I EQG I DHIWWGFCK I CEFAELCRGGCSWI AHVFFDKK
	GNNPYCHHRALKQAQKDIRERFYLKVKAKGNPFDNGEFVIIEEPFNAPLPENDLLHFNSDHIQWPENW
	QNSESAYALAK
PlpY	MNSNQIPNKVATAAQKSDDSSSVLPRQGWQDKQAFIKALIKAKQSLEIAEISNFLT
PIpXY _{fusion}	MTKKYRRVSYAVWEITLKCNLACSHCGSRAGQARTKELSTEEAFNLVRQLADVGIKEVTLIGGEAFMR
	SDWLEIAKAVTEAGMICGMTTGGFGVSLETARKMKEAGIKTVSVSIDGGIPETHDRQRGKKGAWHSAF
	RTMSHLKEVGIYFGCNTQINRLSASEFPIIYERIRDAGARAWQIQLTVPMGNAADNADMLLQPYELLDIY
	PMLARVAKRAKQEGVRIQAGNNIGYYGPYERLLRGSDEWTFWQGCGAGLNTLGIEADGKIKGCPSLP
	TAAYTGGNIRDRPLREIVEQTEELKFNLKAGTEQGTDHMWGFCKTCEFAELCRGGCSWTAHVFFDRR
	GNNPYCHHRALKQAQKDIRERFYLKVKAKGNPFDNGEFVIIEEPFNAPLPENDLLHFNSDHIQWPENW
	QNSESAYALAKMNSNQIPNKVATAAQKSDDSSSVLPRQGWQDKQAFIKALIKAKQSLEIAEISNFLT
His-RhaA	MGSSHHHHHHSODPMKNVKI DTNHVVEAVELI NKVEGGINATGORIDSSVETHCDEEVCGVALYGSV
PhoY	
NIIdA	IN SLANDSINGHT AVVET LAVET AVVET LAVEROVACION DOSTANDANCE ACISNI/SVS/VOL E ATHORI DOVI CA
	WQQCFA HEREKAYGINYGON QINKRAA EEMIL QQUVQA O'SAWQL YEMIGNAYEMIXAMULQ
	PTELLELTPVLATLSKRGKRDKLMVQPGNNIGTPGPTERLLREPISKRKDPAFFRGCGAGINTIGIEAD
	GKVKGCP5LP5EQY1GGNIRER5LRDIYENSKELRFNDINKPEDV1AHMWGDCASCEYAKVCRAGCS
	WIAHVFFGRRGNNPYCHHRALKKAVLGKMERFYLKIPAAGQPFDHGVFELVEEQIKPFDPMDPAHFS
	IAQTQFPAEWLAEEPDLQKSLMLERSMLMLQYVESGIVKQADSPWFDPAKREAIKQGIAIAS
RhaXA _{leader}	MTSLANSGIKLRHRQTYAVWEITLKCNLACSHCGSRAGDSRVNELSTSEALDLVQQMAELGIEDVSLIG
	GEAFLRPDWLIIAAEITRLGMNANMTTGGYGISRGTAKRMKEAGISNVSVSVDGLEATHDKLRGKLGA
	WQQCFKTIEHLRAVGINVGCNTQINKHSATELPMLYQQLVQHGVSAWQIQLTVPMGNAVEHNAMLLQ
	PYELLELYPVLAYLSKRGRKDKLMVQPGNNIGYFGPYERLLREPISRHRDFAFFRGCGAGINTIGIEAD
	GKVKGCPSLPSEQYTGGNIRERSLRDIYENSKELRFNDINKPEDVTAHMWGDCASCEYAKVCRAGCS
	WTAHVFFGRRGNNPYCHHRALKKAVLGKMERFYLKTPAAGQPFDHGVFELVEEQIKPFDPMDPAHFS
	IAQTQFPAEWLAEEPDLQKSLMLERSMLMLQYVESGIVKQADSPWFDPAKREAIKQGIAIAS <i>MKNVKL</i>
	DTNHVVEAVELLNKVE
His ₆ -SUMO-	MGSHHHHHHHSSGLVPRGSASHINLKVKGQDGNEVFFRIKRSTQLKKLMNAYCDRQSVDMTAIAFLF
Rham	DGRRLRAEOTPDELEMEDGDEIDAMLHOTGGHMASMTGGOGGINATGQRIDSSVFTHCDFFVCGVA
His-mCharry200	
Pho Dho	EMYCREDINIKALICA INTERVITINE OSONOFIE ELECTED CALLET A CALLO TRADICIÓN A CALLER A CA
IN Id _{core}	
HIS ₆ -DHFR118-	MGSSHHHHHHSQUPENAMPWNLPADLAWVKRNILNKPVIMGRHTWESIGRPLPGRKNIILSSQPGTD
RhaA _{core}	DRVIWVKSVDEAIAACGDVPEIMVIGGGRVYEQLLPKAQKLYLTHIDAEVEGG <u>INATGQRIDSSVFTHC</u>
	<u>DFFVCGVALYGSVIDPHRFDMLP</u> GDTHYPDYEPDDWERVFSEYHDADAQNSHSYCYEILERRGSRSH
	НННН
His ₆ -MBP-Cterm-	MGSSHHHHHHSSGLVPRGSHNKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKF
RhaA _{core}	PQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIY
	NKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDN
	AGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQ
	PSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATME

	NAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNLGIEGLYFQ
	SGSEFELGAPAGRQACGRIMLKSNRKGG <u>INATGQRIDSSVFTHCDFFVCGVALYGSVIDPHRFDMLP</u>
His ₆ -MBP154-	MGSSHHHHHHSSGLVPRGSHNKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKF
RhaA _{core}	PQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIY
	NKDLLPNPPKTWEEIPGGINATGQRIDSSVFTHCDFFVCGVALYGSVIDPHRFDMLPALDKELKAKGKS
	ALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAE
	AAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLEN
	YLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAA
	SGRQTVDEALKDAQTNSSSNNNNNNNNNLGIEGLYFQSGSEFELGAPAGRQACGRIMLKSNRK
DHFR-Nterm-	MGG <u>INATGQRIDSSVFTHCDFFVCGVALYGSVIDPHRFDMLP</u> QDPENAMPWNLPADLAWVKRNTLNK
RhaA _{core} -His ₆	PVIMGRHTWESIGRPLPGRKNIILSSQPGTDDRVTWVKSVDEAIAACGDVPEIMVIGGGRVYEQLLPKA
	QKLYLTHIDAEVEGDTHYPDYEPDDWERVFSEYHDADAQNSHSYCYEILERRGSRSHHHHHH
His ₆ -SUMO-	MGSHHHHHHHSSGGG <u>INATGQRIDSSVFTHCDFFVCGVALYGSVIDPHRFDMLP</u> LVPRGSASHINLKV
Nterm-RhaA _{core}	KGQDGNEVFFRIKRSTQLKKLMNAYCDRQSVDMTAIAFLFDGRRLRAEQTPDELEMEDGDEIDAMLH
	QTGG
His ₆ -SUMO-	MGSHHHHHHHSSGLVPRGSASHINLKVKGQDGNEVFFRIKRSTQLKKLMNAYCDRQSVDMTAIAFLF
Rha _{core} -N8	DGRRLRAEQTPDELEMEDGDEIDAMLHQTGGHGG <u>INATGQRIDSSVFTHCDFFVCGVALYGSVIDPH</u>
	RFDMLP
His ₆ -SUMO-	MGSHHHHHHHSSGLVPRGSASHINLKVKGQDGNEVFFRIKRSTQLKKLMNAYCDRQSVDMTAIAFLF
Rha _{core} -N24	DGRRLRAEQTPDELEMEDGDEIDAMLHQTGGHH <u>CDFFVCGVALYGSVIDPHRFDMLP</u>
His ₆ -SUMO-	MGSHHHHHHHSSGLVPRGSASHINLKVKGQDGNEVFFRIKRSTQLKKLMNAYCDRQSVDMTAIAFLF
Rha _{core} -N32	DGRRLRAEQTPDELEMEDGDEIDAMLHQTGGH <u>VALYGSVIDPHRFDMLP</u>
His ₆ -SUMO-	MGSHHHHHHHSSGLVPRGSASHINLKVKGQDGNEVFFRIKRSTQLKKLMNAYCDRQSVDMTAIAFLF
Rha _{core} -N24C4	DGRRLRAEQTPDELEMEDGDEIDAMLHQTGGH <u>HCDFFVCGVALYGSVIDPHRF</u>
His ₆ -SUMO-	MGSHHHHHHHSSGLVPRGSASHINLKVKGQDGNEVFFRIKRSTQLKKLMNAYCDRQSVDMTAIAFLF
Rha _{core} -N24C8	DGRRLRAEQTPDELEMEDGDEIDAMLHQTGGH <u>HCDFFVCGVALYGSVID</u>
His ₆ -SUMO-	MGSHHHHHHHSSGLVPRGSASHINLKVKGQDGNEVFFRIKRSTQLKKLMNAYCDRQSVDMTAIAFLF
Rha _{core} -N24C10	DGRRLRAEQTPDELEMEDGDEIDAMLHQTGGH <u>HCDFFVCGVALYGSV</u>

 Table S4. Nucleotide sequences encoding for protein sequences used in this study.

Encoded Protein	Nucleotide Sequence
His ₆ -PlpA3	ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGT
	CTATTGAAAGTGCAAAAGCTTTTTACCAAAGAATGACCGATGATGCATCCTTTCGCACACCATTTG
	AAGCAGAATTATCTAAAGAAGAACGCCAACAACTAATTAAAGACTCTGGCTATGATTTCACTGCCG
	AGGAATGGCAGCAAGCGATGACAGAAATTCAAGCTGCTAGGTCTAATGAGGAATTGAATGAA
	GAACTTGAAGCGATCGCAGGTGGTGCTGTAGCAGCAATGTATGGCGTAGTTTTTCCTTGGGATAA
	TGAATTTCCTTGGCCTAGGTGGGGGGGGATAA
His ₆ -PlpYA3-33	ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCAACTCTAATCAAATACCAAATAAAGTA
	GCCACAGCAGCTCAAAAATCAGATGATTCCAGCTCGGTTTTACCTCGTCAGGGTTGGCAAGACAA
	GCAAGCTTTTATCAAAGCATTAATTAAAGCAAAACAAAGTTTAGAAATTGCTGAAATTAGCAATTTT
	CTGACATTATCTAAAGAAGAACGCCAACAACTAATTAAAGACTCTGGCTATGATTTCACTGCCGAG
	GAATGGCAGCAAGCGATGACAGAAATTCAAGCTGCTAGGTCTAATGAGGAATTGAATGAA
	ACTTGAAGCGATCGATGGTCGTGCTGTAGCAGCAATGTATGGCGTAGTTTTTCCTTGGGATAATG
	AATTTCCTTGGCCTAGGTGGGGGGGGATAA
His ₆ -PlpYA3-45	ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCAACTCTAATCAAATACCAAATAAAGTA
	GCCACAGCAGCTCAAAAATCAGATGATTCCAGCTCGGTTTTACCTCGTCAGGGTTGGCAAGACAA
	GCAAGCTTTTATCAAAGCATTAATTAAAGCAAAACAAAGTTTAGAAATTGCTGAAATTAGCAATTTT
	CTGACATCTGGCTATGATTTCACTGCCGAGGAATGGCAGCAAGCGATGACAGAAATTCAAGCTGC
	TAGGTCTAATGAGGAATTGAATGAAGAAGAACTTGAAGCGATCGAT
	TGTATGGCGTAGTTTTTCCTTGGGATAATGAATTTCCTTGGCCTAGGTGGGGGGGG
His ₆ -PcpA	ATGGGCAGCAGCCATCATCATCATCACCAGCAGCGGCGAGAATCTCTACTTCCAGTCACATAT
	GTCCTCAAATATCTTAGAAAAAGTCAAAGAGTTTTTTGTCAGGCTAGTTAAAGATGACGCTTTCCAA
	TCTCAACTTCAAAATAATTCAATCGACGAAGTTAGAAACATCTTACAAGAAGCTGGCTATATTTTCT
	CAAAAGAAGAATTTGAGACAGCTACGATTGAGCTACTCGATTTAAAAGAACGAGACGAATTTCACG
	AACTGACAGAAGAAGAGTTAGTGACAGCCGTTGGTGGGGTGACTGGGGGTAGCGGGATCTACGG
	ACCCATTCAGGCAATGTACGGAGCAGTGGTAGGAGATCCCAAGCCCGGTAAGGATTGGGGTTGG

	CGCTTTCCAAGTCCGTTACCAAAGCCATCACCCATACCTTCTCCCTGGAAGCCGCCAGTTGACGT
	GCAGCCGATGTATGGGGTAGTTGTCTCAAACGATTCATAA
His ₆ -SUMO-PcpX	ATGGGTAGCCACCACCACCATCATCATAGCAGCGGTTTAGTTCCTCGTGGTTCAGCTAGCCA
	CATCAACCTGAAGGTGAAAGGCCAGGATGGCAACGAGGTGTTCTTCCGCATTAAACGCTCAACCC
	AGCTGAAGAAGCTGATGAACGCGTACTGCGATCGTCAGAGCGTGGATATGACCGCAATTGCGTTC
	CTGTTCGATGGTCGTCGTTTACGTGCAGAACAAACCCCGGACGAACTGGAAATGGAAGATGGCG
	ACGAGATTGATGCCATGCTGCATCAGACCGGTGGCCATATGACTTATCGCAGAACCAGTTATGCC
	GTTTGGGAAATTACCCTAAAGTGTAATTTAGCCTGTAGTCACTGCGGTTCGCGAGCGGGACATAC
	CAGGGCTAAGGAACTCTCGACACAAGAGGCTCTCGATCTCGTCCGGCAGATGGCGGACGTAGGG
	ATTATAGAGGTGACGTTGATTGGCGGCGAGGCATTTCTTCGTCCAGACTGGCTGCAAATTGCAGA
	GGCTATTACTAAGGCGGGGATGCTATGCAGTATGACGACTGGCGGCTATGGCATTTCGCTGGAG
	ACGGCGCGTAAAATGAAGGCAGCAGGCATCGCTTCGGTTTCGGTTTCCATTGATGGGCTCGAAG
	AAACCCACGATCGCCTGCGGGGAAGAAAAGGCTCCTGGCAAGCTGCTTTTAAGACTATGAGCCA
	CCTGCGAGAAGTCGGGATTTTCTTTGGCTGCAACACCCAAATCAACCGTCTTTCTGCGCCGGAAT
	TTCCCCTCATTTACGAACGCATTCGCGACGCTGGCGCTAGGGCTTGGCAAATTCAGCTAACCGTA
	CCGATGGGAAGGGCAGCAGACAATGCCAATATTCTGCTGCAACCTTACGAGTTACTAGATCTCTA
	CCCAATGATAGCCCGCGTAGCCCGTCGGGCACGGCAAGAAGGCGTTCAGATCCAGCCTGGAAAC
	AATATTGGTTATTATGGTCCTTACGAACGGCTCTTACGGGGACGAGGAAGCGATAGCGAATGGGC
	ATTTTGGCAGGGTTGCGCTGCCGGACTCTCAACCTTGGGAATCGAGGCAGACGGCGCAATTAAG
	GGGTGTCCTTCACTGCCAACTTCGGCTTACACCGGCGGAAACATTCGAGAGCATTCGCTGCGGG
	AGATCGTTGAAGAATCAGAGCAACTACGTTTCAATCTCGGCGCGGGAACGTCCCAGGGAACTGCT
	CATCTGTGGGGGTTCTGCCAAACCTGCGAGTTTTCAGAACTGTGTCGTGGGGGGCTGCACTTGGA
	CTGCCCACGTTTTCTTCAACCGTCGAGGAAACAACCCTTACTGCCATCACCGGGCGCTCTTTCAA
	GCAGAGCAAGGCATTCGGGAGCGCGTCGTTCCTAAAGTAGAGGCGCAGGGACTACCTTTTGACA
	ATGGGGAATTCGAGCTAATTGAAGAGCCGATTGATGCTCCTTTACCAGAAAACGATCCCCTGCATT
	TTACATCCGATCTCGTCCAGTGGTCGGCAAGCTGGCAGGAAGAATCCGAGTCGATTGGTGCTGTA
	GTTGATTAA
His ₆ -SUMO-PcpY	ATGGGTAGCCACCACCACCATCATCATAGCAGCGGTTTAGTTCCTCGTGGTTCAGCTAGCCA
	CATCAACCTGAAGGTGAAAGGCCAGGATGGCAACGAGGTGTTCTTCCGCATTAAACGCTCAACCC
	AGCTGAAGAAGCTGATGAACGCGTACTGCGATCGTCAGAGCGTGGATATGACCGCAATTGCGTTC
	CTGTTCGATGGTCGTCGTTTACGTGCAGAACAAACCCCGGACGAACTGGAAATGGAAGATGGCG
	ACGAGATTGATGCCATGCTGCATCAGACCGGTGGCCATATGGTCGAAAATATAGACAACGAGCGA
	GAGAAATCGGCAAATGAAATCGAGCCAGAATCTCTCCTATTGCCCCGTCAAGCTTGGCAAAGTCA
	AATTGCCTATCTAAAAGCGATTTTGAAAGCCAAACAAGCTCTAGATCGAATAGAAAAAAGGTATTT
	GCGTTAA
PcpY	ATGGTCGAAAATATAGACAACGAGCGAGAGAAATCGGCAAATGAAATCGAGCCAGAATCTCTCCT
	ATTGCCCCGTCAAGCTTGGCAAAGTCAAATTGCCTATCTAAAAGCGATTTTGAAAGCCAAACAAGC
	TCTAGATCGAATAGAAAAAAGGTATTTGCGTTAA
His ₆ -SUMO-	ATGGGTAGCCACCACCACCACCATCATCATAGCAGCGGTTTAGTTCCTCGTGGTTCAGCTAGCCA
PcpXYfusion	CATCAACCTGAAGGTGAAAGGCCAGGATGGCAACGAGGTGTTCTTCCGCATTAAACGCTCAACCC
	AGCTGAAGAAGCTGATGAACGCGTACTGCGATCGTCAGAGCGTGGATATGACCGCAATTGCGTTC
	CTGTTCGATGGTCGTCGTTTACGTGCAGAACAAACCCCCGGACGAACTGGAAATGGAAGATGGCG
	ACGAGATTGATGCCATGCTGCATCAGACCGGTGGCCATATGACTTATCGCAGAACCAGTTATGCC
	GTTTGGGAAATTACCCTAAAGTGTAATTTAGCCTGTAGTCACTGCGGTTCGCGAGCGGGACATAC
	CAGGGCTAAGGAACTCTCGACACAAGAGGCTCTCGATCTCGTCCGGCAGATGGCGGACGTAGGG
	ATTATAGAGGTGACGTTGATTGGCGGCGAGGCATTTCTTCGTCCAGACTGGCTGCAAATTGCAGA
	GGCTATTACTAAGGCGGGGATGCTATGCAGTATGACGACTGGCGGCTATGGCATTTCGCTGGAG
	ACGGCGCGTAAAATGAAGGCAGCAGGCATCGCTTCGGTTTCGGTTTCCATTGATGGGCTCGAAG
	AAACCCACGATCGCCTGCGGGGAAGAAAAGGCTCCTGGCAAGCTGCTTTTAAGACTATGAGCCA
	CCTGCGAGAAGTCGGGATTTTCTTTGGCTGCAACACCCAAATCAACCGTCTTTCTGCGCCGGAAT
	TTCCCCTCATTTACGAACGCATTCGCGACGCTGGCGCTAGGGCTTGGCAAATTCAGCTAACCGTA
	CCGATGGGAAGGGCAGCAGACAATGCCAATATTCTGCTGCAACCTTACGAGTTACTAGATCTCTA
	CCCAATGATAGCCCGCGTAGCCCGTCGGGCACGGCAAGAAGGCGTTCAGATCCAGCCTGGAAAC
	AATATTGGTTATTATGGTCCTTACGAACGGCTCTTACGGGGGACGAGGAAGCGATAGCGAATGGGC
	ATTTTGGCAGGGTTGCGCTGCCGGACTCTCAACCTTGGGAATCGAGGCAGACGGCGCAATTAAG
	GGGTGTCCTTCACTGCCAACTTCGGCTTACACCGGCGGAAACATTCGAGAGCATTCGCTGCGGG
	AGATCGTTGAAGAATCAGAGCAACTACGTTTCAATCTCGGCGCGGGAACGTCCCAGGGAACTGCT
	CATCTGTGGGGGGTTCTGCCAAACCTGCGAGTTTTCAGAACTGTGTCGTGGGGGGCTGCACTTGGA
	CTGCCCACGTTTTCTTCAACCGTCGAGGAAACAACCCTTACTGCCATCACCGGGCGCTCTTTCAA
	GCAGAGCAAGGCATTCGGGAGCGCGTCGTTCCTAAAGTAGAGGCGCAGGGACTACCTTTTGACA

	TTACATCCGATCTCGTCCAGTGGTCGGCAAGCTGGCAGGAAGAATCCGAGTCGATTGGTGCTGTA
	GTTGATTAAATGGTCGAAAATATAGACAACGAGCGAGAGAAATCGGCAAATGAAATCGAGCCAGA
	ATCTCTCCTATTGCCCCGTCAAGCTTGGCAAAGTCAAATTGCCTATCTAAAAGCGATTTTGAAAGC
	CAAACAAGCTCTAGATCGAATAGAAAAAAGGTATTTGCGTTAA
PlpX	ATGACTAAAAAATACAGACGAGTTAGTTATGCAGTTTGGGAAATTACCTTGAAATGCAATCTAGCTT
	GTAGTCACTGTGGTTCGAGAGCAGGCAGGCAGGCAAGGAACCAAGGAACTATCTACAGAAGAAGCATTTT
	TATGCGCTCTGATTGGCTAGAAATTGCCAAGGCTGTTACTGAGGCAGGGATGATCTGCGGTATGA
	CTACAGGTGGATTTGGTGTCAGTTTGGAAACTGCCAGAAAAATGAAAGAAGCTGGAATTAAAACA
	GTTTCTGTATCTATCGATGGTGGCATACCAGAAACCCACGATCGCCAGCGAGGGAAAAAAGGTGC
	TTGGCATTCTGCTTTTAGAACCATGAGCCATCTAAAAGAAGTCGGCATCTATTTTGGCTGCAATAC
	CCAGATAAACCGTTTATCTGCCTCTGAATTCCCAATAATTTACGAACGA
	AAGAGCTTGGCAGATTCAATTAACTGTACCTATGGGTAATGCGGCAGATAATGCAGACATGTTATT
	GCAACCATACGAACTATTAGATATTTATCCCATGTTAGCTCGTGTTGCTAAACGAGCTAAACAGGA
	GradioandardoantingGradgen ingegoadedet interaction and interaction
	GATGGCAAAATTAAAGGTTGTCCTTCTTTACCTACGGCTGCTTATACGGGCGGTAATATCCGCGAT
	CGCCCTTTAAGAGAAATAGTCGAACAGACTGAAGAGCTTAAATTTAATCTGAAGGCTGGGACTGAA
	CAGGGCACAGACCACATGTGGGGGATTTTGTAAAACCTGTGAATTTGCTGAACTCTGTCGAGGTGG
	TTGTTCTTGGACGGCTCATGTCTTCTTTGATCGCCGTGGGAATAATCCCTACTGCCATCATCGTGC
	TTTGAAACAGGCACAAAAAGACATCAGAGAAAGATTCTATTTAAAAGTAAAAGCAAAAGGGAATCC
	TTTTGATAATGGGGAATTTGTCATTATAGAAGAACCTTTCAACGCACCTTTGCCAGAGAACGATTT
	GCTTCATTTTAATAGCGATCACATTCAGTGGCCAGAAAACTGGCAAAATTCTGAATCTGCTTACGC
	TTTAGCAAAGTAA
DlpV	
FIPT	
	AGTTAGAAATIGCTGAAATIAGCAATTTCTGACATAA
PIpXY _{fusion}	ATGACTAAAAAATACAGACGAGTTAGTTATGCAGTTTGGGAAATTACCTTGAAATGCAATCTAGCTT
	GTAGTCACTGTGGTTCGAGAGCAGGGCAGGCAAGAACCAAGGAACTATCTACAGAAGAAGCTTTT
	AATCTGGTTCGGCAACTAGCCGATGTAGGAATCAAAGAGGTTACTCTAATCGGTGGCGAAGCCTT
	TATGCGCTCTGATTGGCTAGAAATTGCCAAGGCTGTTACTGAGGCAGGGATGATCTGCGGTATGA
	CTACAGGTGGATTTGGTGTCAGTTTGGAAACTGCCAGAAAAATGAAAGAAGCTGGAATTAAAACA
	GTTTCTGTATCTATCGATGGTGGCATACCAGAAACCCACGATCGCCAGCGAGGGAAAAAAGGTGC
	TIGGCATICIGCTITTAGAACCATGAGCCATCTAAAAGAAGTCGGCATCTATTTIGGCIGCAATAC
	GCAACCATACGAACTATTAGATATTATCCCATGTTAGCTCGTGTTGCTAAACGAGCTAAACGAGGA
	AGGIGIICGCATACAGGCGGGAAATAATATIGGCTATTATGGCCCTTATGAAAGACIGCTGCGIG
	GTAGTGATGAATGGACATTTTGGCAGGGTTGCGGAGCGGGTTTAAATACCTTGGGTATCGAAGCT
	GATGGCAAAATTAAAGGTTGTCCTTCTTTACCTACGGCTGCTTATACGGGCGGTAATATCCGCGAT
	CGCCCTTTAAGAGAAATAGTCGAACAGACTGAAGAGCTTAAATTTAATCTGAAGGCTGGGACTGAA
	CAGGGCACAGACCACATGTGGGGGATTTTGTAAAACCTGTGAATTTGCTGAACTCTGTCGAGGTGG
	TTGTTCTTGGACGGCTCATGTCTTCTTTGATCGCCGTGGGAATAATCCCTACTGCCATCATCGTGC
	TTTGAAACAGGCACAAAAAGACATCAGAGAAAGATTCTATTTAAAAGTAAAAGCAAAAGGGAATCC
	TTTTGATAATGGGGAATTTGTCATTATAGAAGAACCTTTCAACGCACCTTTGCCAGAGAACGATTT
	GCTTCATTTTAATAGCGATCACATTCAGTGGCCAGAAAACTGGCAAAATTCTGAATCTGCTTACGC
	TTTAGCAAAGATGAACTCTAATCAAATACCAAATAAAGTAGCCACAGCAGCTCAAAAATCAGATGA
HIS ₆ -RhaA	A I G G G C A G C A I C A I C A I C A I C A C A C A C A
	AAACCATGTTGTTGAGGCTGTAGAGCTACTAAACAAAGTTGAAGGCGGGATTAACGCTACCGGTC
	AGCGTATCGACTCCTCGGTTTTTACCCACTGTGACTTTTTCGTTTGTGGTGTAGCGCTGTACGGCA
	GCGTAATTGATCCTCATCGTTTCGATATGTTGCCTTAA
RhaX	ATGACATCGCTTGCAAACTCCGGCATTAAACTCCGACACCGCCAAACCTATGCTGTATGGGAAAT
	CACCCTAAAATGCAATCTGGCTTGCAGCCATTGTGGTTCGAGGGCAGGCGATTCACGTGTAAACG
	AGCTGAGTACCAGCGAGGCGCTGGATCTGGTGCAGCAAATGGCTGAGCTTGGTATCGAGGATGT
	TTCTCTGATCGGCGGTGAGGCATTTTTGCGACCAGACTGGTTAATTATTGCTGCAGAAATTACCCG
	TCTTGGCATGAATGCCAACATGACGACCGGGGGGCTACGGGATATCACGCGGTACGGCAAAACGG
	ATGAAAGAAGCGGGTATCAGTAACGTTTCGGTATCGGTAGATGGCCTTGAGGCTACGCACGATAA

	GCTACGTGGTAAGCTGGGTGCCTGGCAGCAATGTTTTAAGACGATAGAACATTTACGCGCCGTGG
	GGATCAATGTTGGCTGCAATACGCAGATCAACAAGCACTCCGCTACCGAGTTGCCCATGTTGTAT
	CAGCAATTAGTCCAGCATGGCGTGTCGGCCTGGCAGATACAGCTTACCGTACCAATGGGCAATG
	CGGTAGAGCATAACGCTATGTGCTGCAGCCTTACGAGCTACTGGAGTTATATCCAGTGCTGCGG
	TATCTGTCTAAACCCCGCCGTAAGCATAAGCTTATGCTGCAGCCCGCGTAATAACATCGCTGCTTACTT
	TACCTICCGAGCAATACACTGGCGGTAATATCCGTGAACGTAGCTTGCGCGATATTATGAAAAC
	AGTAAAGAGCTACGATTTAACGATATCAATAAGCCTGAAGATGTCACGGCCCATATGTGGGGGCGA
	TTGCGCAAGTTGTGAATACGCCAAGGTCTGCCGCGCTGGCTG
	TTGGTCGGCGCGGGAATAACCCTTATTGCCATCACCGGGCATTGAAGAAAGCCGTGCTGGGCAA
	GATGGAGCGTTTTTACCTGAAAACGCCTGCTGCCGGCCAGCCA
	TGGTTGAGGAGCAGATTAAGCCATTTGACCCGATGGACCCGGCACACTTTAGTATTGCCCAAACA
	CAGTTTCCAGCTGAGTGGTTGGCGGAAGAACCTGATCTGCAGAAAAGCCTTATGCTGGAGAGAAG
	TATGCTGATGCTGCAGTACGTTGAAAGCGGTATAGTCAAACAGGCCGACTCGCCATGGTTTGATC
	CCGCTAAGCGTGAGGCGATAAAACAGGGTATCGCAATAGCCAGCTAG
RhaXA	
i thay of tleader	
	1C11GGCA1GAA1GCCAACA1GACGACCGGGGGGC1ACGGGA1A1CACGCGG1ACGGCAAAACGG
	ATGAAAGAAGCGGGTATCAGTAACGTTTCGGTATCGGTAGATGGCCTTGAGGCTACGCACGATAA
	GCTACGTGGTAAGCTGGGTGCCTGGCAGCAATGTTTTAAGACGATAGAACATTTACGCGCCGTGG
	GGATCAATGTTGGCTGCAATACGCAGATCAACAAGCACTCCGCTACCGAGTTGCCCATGTTGTAT
	CAGCAATTAGTCCAGCATGGCGTGTCGGCCTGGCAGATACAGCTTACCGTACCAATGGGCAATG
	CGGTAGAGCATAACGCTATGTTGCTGCAGCCTTACGAGCTACTGGAGTTATATCCAGTGCTGGCG
	TATCTGTCTAAACGCGGCCGTAAGGATAAACTTATGGTGCAGCCGGGTAATAACATCGGTTACTTT
	GGTCCGTACGAGCGCCTGTTGCGGGAGCCGATTTCCCGTCACCGCGACTTTGCGTTTTTCCGCG
	GCTGTGGTGCGGGCATAAATACCATAGGCATAGAAGCGGACGGCAAAGTAAAAGGTTGCCCTTCT
	TTACCTTCCGAGCAATACACTGGCGGTAATATCCGTGAACGTAGCTTGCGCGATATTTATGAAAAC
	AGTAAAGAGCTACGATTTAACGATATCAATAAGCCTGAAGATGTCACGGCCCATATGTGGGGGCGA
	TIGCGCAAGTIGIGAATACGCCAAGGTCIGCCGCGCCGCCGCCGCCAGTIGGACAGCTCATGTCTTTT
	TATECTGATECTGCAGTACGTTGAAAGCGGTATAGTCAAACAGGCCGACTCGCCATGGTTTGATC
	CCGCTAAGCGTGAGGCGATAAAACAGGGTATCGCAATAGCCAGCATGAAAAACGTAAAACTTGAT
	ACAAACCATGTTGTTGAGGCTGTAGAGCTACTAAACAAAGTTGAATAG
His₀-SUMO-	ATGGGTAGCCACCACCACCATCATCATAGCAGCGGTTTAGTTCCTCGTGGTTCAGCTAGCCA
Rha _{core}	CATCAACCTGAAGGTGAAAGGCCAGGATGGCAACGAGGTGTTCTTCCGCATTAAACGCTCAACCC
	AGCTGAAGAAGCTGATGAACGCGTACTGCGATCGTCAGAGCGTGGATATGACCGCAATTGCGTTC
	CTGTTCGATGGTCGTCGTTTACGTGCAGAACAAACCCCGGACGAACTGGAAATGGAAGATGGCG
	ACGAGATTGATGCCATGCTGCATCAGACCGGTGGCCATATGGCTAGCATGACTGGTGGACAGGG
	CGGGATTAACGCIACCGGTCAGCGTATCGACICCICGGTTTTACCCACIGIGACTTTTCGTTIG
	TGGTGTAGCGCTGTACGGCGCGCGTAATTGATCCTCATCGTTCGCATATGTGCCTTGACAAAT
Llia mCharm(100	
RIS6-moneny 109-	
Rna _{core}	ACAT GGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCA
	CGAGTICGAGAICGAGGCGAGGGCCGAGGGCCCCCCTACGAGGGCACCCCAGACCGCCAAGC
	GAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTAC
	GGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCG
	AGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGG
	ACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTC
	CGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCC
	CGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTA
	CGACGCTGAGGTCAAGACCACCTACAAGGCCCAAGAAGCCCGTGCAGCTGCCCGGCGGCGGCAT
	TAACGCTACCGGTCAGCGTATCGACTCCTCGGTTTTTACCCACTGTGACTTTTTCGTTTGTGGTGT
	AGCGCTGTACGGCAGCGTAATTGATCCTCATCGTTTCGATATGTTGCCTGGGGGGGG
	TCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGC

	GCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGGAATTGAAGCTTAGATCTT
	GA
His ₆ -DHFR118-	ATGGGCAGCAGCCATCACCATCATCACCACAGCCAGGATCCGGAGAATGCCATGCCATGGAATC
RhaA _{core}	TGCCTGCTGATCTTGCGTGGGTGAAACGCAATACCCTGAACAAACCGGTTATCATGGGGCGCCAT
	ACCTGGGAAAGCATTGGCCGTCCTTTGCCAGGTCGGAAGAACATCATCCTGAGCAGTCAACCGG
	GCACAGATGACCGTGTCACGTGGGTCAAATCCGTGGATGAAGCGATTGCAGCATGTGGCGATGT
	TCCGGAGATCATGGTGATTGGCGGAGGTCGCGTATACGAACAGCTGTTACCGAAAGCGCAGAAA
	CTCTATCTGACTCACATTGACGCCGAAGTAGAAGGCGGGATTAACGCTACCGGTCAGCGTATCGA
	CTCCTCGGTTTTTACCCACTGTGACTTTTTCGTTTGTGGTGTAGCGCTGTACGGCAGCGTAATTGA
	TCCTCATCGTTTCGATATGTTGCCTGGGGATACCCATTATCCGGACTATGAACCCGACGATTGGG
	AACGCGTGTTTAGCGAGTATCACGATGCTGATGCCCAGAACTCGCATTCGTACTGCTACGAGATT
	CTGGAACGTCGTGGTTCACGCTCTCACCATCATCACCACCATTAA
His ₆ -MBP-Cterm-	ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATAATA
RhaA _{core}	AAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAAGGCTATAACGGTCTCGCTGAA
	GTCGGTAAGAAATTCGAGAAAGATACCGGAATTAAAGTCACCGTTGAGCATCCGGATAAACTGGA
	AGAGAAATTCCCACAGGTTGCGGCAACTGGCGATGGCCCTGACATTATCTTCTGGGCACACGACC
	GCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCTGAAATCACCCCGGACAAAGCGTTCCAGGAC
	AAGCTGTATCCGTTTACCTGGGATGCCGTACGTTACAACGGCAAGCTGATTGCTTACCCGATCGC
	TGTTGAAGCGTTATCGCTGATTTATAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAG
	AGATCCCGGCGCTGGATAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAACCTGCA
	AGAACCGTACTTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTATGCGTTCAAGTATGAAAACG
	GCAAGTACGACATTAAAGACGTGGGCGTGGATAACGCTGGCGCGAAAGCGGGTCTGACCTTCCT
	GGTTGACCTGATTAAAAACAAACACATGAATGCAGACACCGATTACTCCATCGCAGAAGCTGCCTT
	TAATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGTCCAACATCGACACCAGC
	AAAGTGAATTATGGTGTAACGGTACTGCCGACCTTCAAGGGTCAACCATCCAAACCGTTCGTT
	CGTGCTGAGCGCAGGTATTAACGCCGCCAGTCCGAACAAAGAGCTGGCAAAAGAGTTCCTCGAA
	AACTATCTGCTGACTGATGAAGGTCTGGAAGCGGTTAATAAAGACAAACCGCTGGGTGCCGTAGC
	GCTGAAGTCTTACGAGGAAGAGTTGGCGAAAGATCCACGTATTGCCGCCACTATGGAAAACGCCC
	AGAAAGGTGAAATCATGCCGAACATCCCGCAGATGTCCGCTTTCTGGTATGCCGTGCGTACTGCG
	GTGATCAACGCCGCCAGCGGTCGTCAGACTGTCGATGAAGCCCTGAAAGACGCGCAGACTAATT
	CGAGCTCGAACAACAACAACAATAACAATAACAACAACCACCTCGGGATCGAGGGACTGTACTTCCAG
	TCAGGATCCGAATTCGAGCTCGGCGCGCCTGCAGGTCGACAAGCTTGCGGCCGCATAATGCTTA
	AGTCGAACAGAAAGGGCGGGATTAACGCTACCGGTCAGCGTATCGACTCCTCGGTTTTTACCCAC
	TGTGACTTTTTCGTTTGTGGTGTAGCGCTGTACGGCAGCGTAATTGATCCTCATCGTTTCGATATG
	TTGCCTTAA
His ₆ -MBP154-	ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATAATA
RhaA _{core}	AAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAAGGCTATAACGGTCTCGCTGAA
	GTCGGTAAGAAATTCGAGAAAGATACCGGAATTAAAGTCACCGTTGAGCATCCGGATAAACTGGA
	AGAGAAATTCCCACAGGTTGCGGCAACTGGCGATGGCCCTGACATTATCTTCTGGGCACACGACC
	GCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCTGAAATCACCCCGGACAAAGCGTTCCAGGAC
	AAGCTGTATCCGTTTACCTGGGATGCCGTACGTTACAACGGCAAGCTGATTGCTTACCCGATCGC
	TGTTGAAGCGTTATCGCTGATTTATAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAG
	AGATCCCGGGCGGGATTAACGCTACCGGTCAGCGTATCGACTCCTCGGTTTTTACCCACTGTGAC
	TTTTTCGTTTGTGGTGTAGCGCTGTACGGCAGCGTAATTGATCCTCATCGTTTCGATATGTTGCCT
	GCGCTGGATAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAACCTGCAAGAACCGT
	ACTTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTATGCGTTCAAGTATGAAAACGGCAAGTAC
	GACATTAAAGACGTGGGCGTGGATAACGCTGGCGCGAAAGCGGGTCTGACCTTCCTGGTTGACC
	TGATTAAAAACAAACACATGAATGCAGACACCGATTACTCCATCGCAGAAGCTGCCTTTAATAAAG
	GCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGTCCAACATCGACACCAGCAAAGTGAA
	TTATGGTGTAACGGTACTGCCGACCTTCAAGGGTCAACCATCCAAACCGTTCGTT
	GCGCAGGTATTAACGCCGCCAGTCCGAACAAAGAGCTGGCAAAAGAGTTCCTCGAAAACTATCTG
	CTGACTGATGAAGGTCTGGAAGCGGTTAATAAAGACAAACCGCTGGGTGCCGTAGCGCTGAAGT
	CTTACGAGGAAGAGTTGGCGAAAGATCCACGTATTGCCGCCACTATGGAAAACGCCCAGAAAGGT
	GAAATCATGCCGAACATCCCGCAGATGTCCGCTTTCTGGTATGCCGTGCGTACTGCGGTGATCAA
	CGCCGCCAGCGGTCGTCAGACTGTCGATGAAGCCCTGAAAGACGCGCAGACTAATTCGAGCTCG
	AACAACAACAACAATAACAATAACAACAACCTCGGGATCGAGGGACTGTACTTCCAGTCAGGATC
	CGAATTCGAGCTCGGCGCGCCTGCAGGTCGACAAGCTTGCGGCCGCATAATGCTTAAGTCGAAC
	AGAAAGTAA
DHFR-Nterm-	ATGGGCGGGATTAACGCTACCGGTCAGCGTATCGACTCCTCGGTTTTTACCCACTGTGACTTTTT
RhaA _{core} -His ₆	CGTTTGTGGTGTAGCGCTGTACGGCAGCGTAATTGATCCTCATCGTTTCGATATGTTGCCTCAGG

	ATCCGGAGAATGCCATGCCATGGAATCTGCCTGCTGATCTTGCGTGGGTGAAACGCAATACCCTG
	AACAAACCGGTTATCATGGGGCGCCATACCTGGGAAAGCATTGGCCGTCCTTTGCCAGGTCGGA
	AGAACATCATCCTGAGCAGTCAACCGGGCACAGATGACCGTGTCACGTGGGTCAAATCCGTGGA
	TGAAGCGATTGCAGCATGTGGCGATGTTCCGGAGATCATGGTGATTGGCGGAGGTCGCGTATAC
	GAACAGCTGTTACCGAAAGCGCAGAAACTCTATCTGACTCACATTGACGCCGAAGTAGAAGGGGA
	TACCCATTATCCGGACTATGAACCCGACGATTGGGAACGCGIGTTTAGCGAGTATCACGATGCIG
	ATGCCCAGAACTCGCATTCGTACTGCTACGAGATTCTGGAACGTCGTGGTTCACGCTCTCACCAT
HIS6-SUIVIO-	ATGGGTAGCCACCACCATCATCATCATAGCAGCGGTGGCGGGGTTAACGCTACCGGTCAGC
Nterm-RhaA _{core}	GTATCGACTCCTCGGTTTTTACCCACTGTGACTTTTTCGTTTGTGGTGTAGCGCTGTACGGCAGCG
	TAATTGATCCTCATCGTTTCGATATGTTGCCTTTAGTTCCTCGTGGTTCAGCTAGCCACATCAACCT
	GAAGGTGAAAGGCCAGGATGGCAACGAGGTGTTCTTCCGCATTAAACGCTCAACCCAGCTGAAG
	AAGCTGATGAACGCGTACTGCGATCGTCAGAGCGTGGATATGACCGCAATTGCGTTCCTGTTCGA
	TGGTCGTCGTTTACGTGCAGAACAAACCCCGGACGAACTGGAAATGGAAGATGGCGACGAGATT
	GATGCCATGCTGCATCAGACCGGTGGC
His-SUMO-	ATGGGTAGCCACCACCACCATCATCATCAGCGGGTTTAGTTCCTCGTGGTTCAGCTAGCCA
Rha N8	
	ACGAGATIGATGCCATGCTGCATCAGACCGGTGGCCATGGCGGGGATTAACGCTACCGGTCAGCG
	TATCGACTCCTCGGTTTTTACCCACTGTGACTTTTCGTTGTGGTGTAGCGCTGTACGGCAGCGT
	AATTGATCCTCATCGTTTCGATATGTTGCCT
His₀-SUMO-	ATGGGTAGCCACCACCACCATCATCATAGCAGCGGTTTAGTTCCTCGTGGTTCAGCTAGCCA
Rha _{core} -N24	CATCAACCTGAAGGTGAAAGGCCAGGATGGCAACGAGGTGTTCTTCCGCATTAAACGCTCAACCC
	AGCTGAAGAAGCTGATGAACGCGTACTGCGATCGTCAGAGCGTGGATATGACCGCAATTGCGTTC
	CTGTTCGATGGTCGTCGTTTACGTGCAGAACAAACCCCGGACGAACTGGAAATGGAAGATGGCG
	ACGAGATTGATGCCATGCTGCATCAGACCGGTGGCCATCACTGTGACTTTTTCGTTTGTGGTGTA
	GCGCTGTACGGCAGCGTAATTGATCCTCATCGTTTCGATATGTTGCCT
His₅-SUMO-	ATGGGTAGCCACCACCACCATCATCATAGCAGCGGTTTAGTTCCTCGTGGTTCAGCTAGCCA
Rham-N32	
	AGCTGAAGAAGCTGATGAACGCGTACTGCGATCGTCAGAGCGTGGATATGACCGCAATTGCGTTC
His ₆ -SUMO-	ATGGGTAGCCACCACCACCATCATCATAGCAGCGGTTTAGTTCCTCGTGGTTCAGCTAGCCA
Rha _{core} -N24C4	CATCAACCTGAAGGTGAAAGGCCAGGATGGCAACGAGGTGTTCTTCCGCATTAAACGCTCAACCC
	AGCTGAAGAAGCTGATGAACGCGTACTGCGATCGTCAGAGCGTGGATATGACCGCAATTGCGTTC
	CTGTTCGATGGTCGTCGTTTACGTGCAGAACAAACCCCGGACGAACTGGAAATGGAAGATGGCG
	ACGAGATTGATGCCATGCTGCATCAGACCGGTGGCCATCACTGTGACTTTTTCGTTTGTGGTGTA
	GCGCTGTACGGCAGCGTAATTGATCCTCATCGTTTC
Hise-SUMO-	ATGGGTAGCCACCACCACCATCATCATAGCAGCGGTTTAGTTCCTCGTGGTTCAGCTAGCCA
RhaN24C8	
HIS6-SUMO-	AIGGGIAGCUACCACCACCAICAICAICAIAGCAGCGGTTTAGTTCCTCGTGGTTCAGCTAGCCA
Rha _{core} -N24C10	CATCAACCTGAAGGTGAAAGGCCAGGATGGCAACGAGGTGTTCTTCCGCATTAAACGCTCAACCC
	AGCTGAAGAAGCTGATGAACGCGTACTGCGATCGTCAGAGCGTGGATATGACCGCAATTGCGTTC
	CTGTTCGATGGTCGTCGTTTACGTGCAGAACAAACCCCCGGACGAACTGGAAATGGAAGATGGCG
	ACGAGATTGATGCCATGCTGCATCAGACCGGTGGCCATCACTGTGACTTTTTCGTTTGTGGTGTA
	GCGCTGTACGGCAGCGTA

Supplementary References

- 1 Scott, T. A. *et al.* Widespread microbial utilization of ribosomal β-amino acid-containing peptides and proteins. *Chem* **8**, 2659-2677, (2022).
- 2 Jumper, J. et al. Highly accurate protein structure prediction with AlphaFold. Nature 596, 583-589, (2021).
- 3 Varadi, M. *et al.* AlphaFold protein structure database: Massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Res.* **50**, 439-444, (2021).
- 4 Morinaka, B. I. *et al.* Natural noncanonical protein splicing yields products with diverse β-amino acid residues. *Science* **359**, 779-782, (2018).
- 5 Kitagawa, M. *et al.* Complete set of ORF clones of *Escherichia coli* ASKA library (a complete set of *E. coli* K-12 ORF archive): Unique resources for biological research. *DNA Res.* **12**, 291-299, (2005).
- 6 Vagstad, A. L. *et al.* Mechanistic insights into post-translational α-keto-β-amino acid formation by a radical S-adenosyl methionine peptide splicease. *Submitted*, (2024).
- Robert, X. & Gouet, P. Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res.* 42, 320-324, (2014).