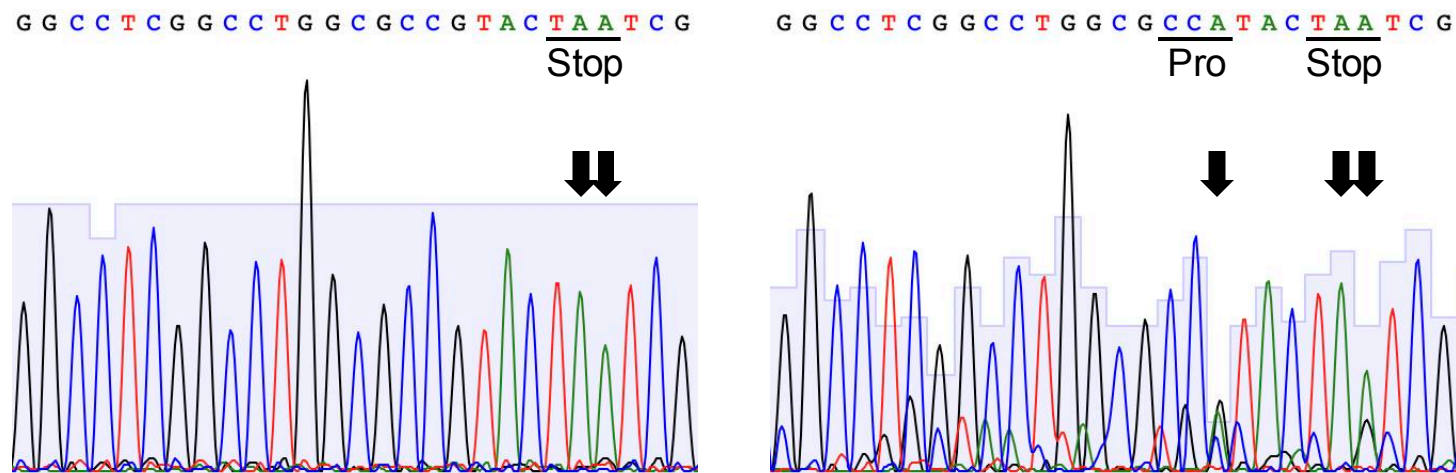


**Table S1.** Oligonucleotides used in this study

<b>Primer</b>	<b>Sequence (5' – 3')</b>
dCas9-Fw	ACGGCGTCAGAGAAGGGAGCGGACA
dCas9-Re	GAGCCGCCGCCGCCGTCGCCGCCGAG
PmCDA1-Fw	CTCGGCGGCGACGGCGGCGGCGGCTC
PmCDA1-Re	TACCCGGGGATCCTCTAGAAAGCTTTCAGGCGACGAGCAG
ssDNA-actI-ORF2	CGGTTGGTAGGATCGACGGCACCAGTACGGCGCCAGGCCGGTTTTAGAG CTAGAAATAGA
ssDNA-lbpA	CGGTTGGTAGGATCGACGGCCCAGTCCTTCGGCATCAACGGTTTTAGAG CTAGAAATAGA
ssDNA-ladA5	CGGTTGGTAGGATCGACGGCCCAGTACGCCCGCTTCGCGGGTTTTAGAG CTAGAAATAGC

**Table S2.** DNA sequences of codon-optimized genes for the construction of the pLK101 vector

Name	Sequence
<i>PmCDAl<sub>str</sub></i>	ATGACCGACGCCGAGTACGTCCGCATCCACGAGAAGCTGGACATCTACA CCTTCAAGAAGCAGTTCTTCAACAACAAGAAGTCCGTCAGCCACCGCTG CTACGTCCTGTTTCGAGCTGAAGCGCCGGGGCGAGCGCCGCGCCTGCTTC TGGGGCTACGCCGTCAACAAGCCGCAGTCCGGCACCGAGCGGGGCATCC ACGCCGAGATCTTCTCCATCCGCAAGGTCGAGGAGTACCTGCGCGACAA CCCGGGCCAGTTCACGATCAACTGGTACTCCAGCTGGTCCCCGTGCGCC GACTGCGCCGAGAAGATCCTCGAGTGGTACAACCAGGAGCTGCGCGGCA ACGGCCACACGCTGAAGATCTGGGCCTGCAAGCTGTACTACGAGAAGAA CGCCCGCAACCAGATCGGCCTGTGGAACCTCCGCGACAACGGCGTCGGC CTGAACGTCATGGTGTCCGAGCACTACCAGTGCTGCCGCAAGATCTTCA TCCAGTCCTCGCACAACCAGCTGAACGAGAACCGCTGGCTCGAGAAGAC CCTGAAGCGGGCCGAGAAGCGCCGCTCCGAGCTGTCCATCATGATCCAG GTCAAGATCCTGCACACCACCAAGTCGCCGGCCGTC
<i>UGI<sub>str</sub></i>	ATGACCAACCTGTCCGACATCATCGAGAAGGAGACCGGCAAGCAGCTGG TCATCCAGGAGTCCATCCTGATGCTCCCCGAGGAGGTCGAGGAGGTCAT CGGCAACAAGCCCAGTCCGACATCCTGGTCCACACCGCCTACGACGAG TCCACCGACGAGAACGTGATGCTGCTGACCTCCGACGCGCCCGAGTACA AGCCGTGGGCGCTCGTGATCCAGGACTCCAACGGCGAGAACAAGATCAA GATGCTG
<i>LVA<sub>str</sub></i>	CTCGTCGCC
Linker region (GS linker, SH3 domain and 3×FLAG tag)	GGCGGGCGGGCTCCGGCGGGCGGGCAGCGCCGAGTACGTGCGCGCCC TGTTTCGACTTCAACGGCAACGACGAGGAGGACCTGCCGTTCAAGAAGGG CGACATCCTGCGCATCCGCGACAAGCCCGAGGAGCAGTGGTGGAAACGCC GAGGACTCCGAGGGCAAGCGCGGCATGATCCCGGTGCCGTACGTGAGA AGTAC TCCGGC GACTACAAGGACCACGACGGGGACTACAAGGACCACGA CATCGACTACAAGGACGACGACGACAAGTCCCGCCTCGAGTCGGGCGAC TACAAGGACCACGACGGGGACTACAAGGACCACGACATCGACTACAAGG ACGACGACGACAAGAGCCGC



**Figure S1**

Sanger sequencing chromatograms of the target region with the predicted C to T mutation in the *mactI-ORF2* strains. Mutated bases are indicated by black arrows.

SCO3413	1	MSYSVGQVAGFAGVTVRTLHHYDDIGLLVPSE	SHAGHRRYS	DADLDRLQQILFYRELGF	60		
SLA_TipA	1	MGYSVGQVAGFAGVTVRTLHHYDEIGLLSPSGRSGAGHRRYD	DADLDRLQRILFYRELGF		60		
SCO3413	61	PLDEVAALLDDPAA	DPRAHLRRQH	ELLSARIGKLQKMAAAVEQAMEARS	120		
SLA_TipA	61	PLDEVAVLLDDPES	DPREHLRRQH	ALLSDRIARLQQMAKAVEHAMEAKK	120		
SCO3413	121	EVFGDFDPDQYEE	EVRRWGN	TDAYRQSKEKTAS	YTKEDWQRIQDEADELTRRFVALMDA	180	
SLA_TipA	121	EVFGDKDPEQYAE	EAERRWGGTEAYAES	QRRAAAYTKADWQRIQDEVADWGGRYAALVAA	180		
SCO3413	181	GEPADSEGAMDAAED	HRQGTIARNHYDCGYEMHT	CLGEMYVSDERF	TRNIDA	AKPGLAAYM	240
SLA_TipA	181	GEPADGEAAMD	LAEEHRRHICDRYYECGYEMHV	CLGEMYVAD	ERFKAFYD	GMGAGVAEHL	240
SCO3413	241	RDA	ILANAVR	HTP			253
SLA_TipA	241	RDS	ITANAVR	KA-			252

## Figure S2

Sequence alignment of SCO3413 (TipA) from *S. coelicolor* A3(2) with putative TipA (SLA\_TipA) from *S. lavendulae* FRI-5. Identical amino acid residues are highlighted in black.

		*				*				*	
KSL	145	RGEHGLALAA	154	AAVGRALATTRHPF	304	TATARPA	344				
KS1	166	TVDTACSSSL	175	VEAHGTGTRLGDPI	316	NIGHTQA	349				
KS2	166	TVDTACSSSL	175	VEAHGTGTRLGDPI	316	NIGHTQA	349				
KS3	166	TVDTACSSSL	175	VEAHGTGTTLGDPI	316	NIGHTQA	349				
KS4	167	TVDTACSSSL	176	VEAHGTGTTLGDPI	317	NIGHTQA	350				
KS5	166	TVDTACSSSL	175	VEAHGTGTTLGDPI	316	NIGHTQA	349				
KS6	165	TVDTACSSSL	174	VEAHGTGTRLGDPI	315	NIGHTQA	348				
KS7	166	TIDTACSSSL	175	VEAHGTGTTLGDPI	316	NLGHTQA	349				
KS8	167	TVDTACSSSL	176	VEAHGTGTTLGDPI	317	NIGHTQA	350				
KS9	166	TVDTACSSSL	175	VEAHGTGTALGDPI	316	NIGHTQA	349				
KS10	165	TVDTACSSSL	174	VEAHGTGTS LGDPI	315	NIGHTQA	348				
KS11	166	TVDTACSSSL	175	VEAHGTGTTLGDPI	316	NIGHTQA	349				
KS12	165	TVDTACSSSL	174	VEAHGTGTTLGDPI	315	NIGHTQA	348				
KS13	166	TVDTACSSSL	175	VEGHGTGTRLGDPI	316	NIGHTQA	349				
Consensus		DXXCSSXL		HGTGT		H					

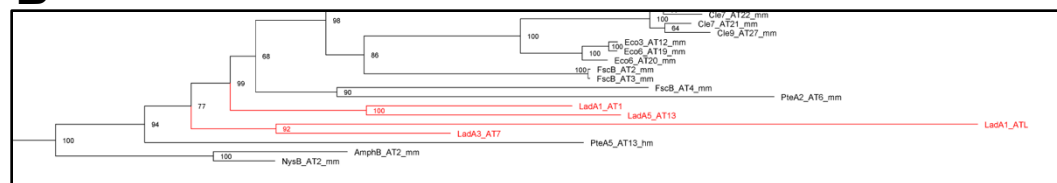
**Figure S3**

Sequence alignment of the conserved motifs in the KS domain core regions from LadA PKSs. The numbers indicate amino acid positions within each domain. Black and gray boxes in the alignment indicate positions at which the same amino acid is found and at least 70% of the amino acids match, respectively. Asterisks indicate the conserved catalytic triad of C-H-H.

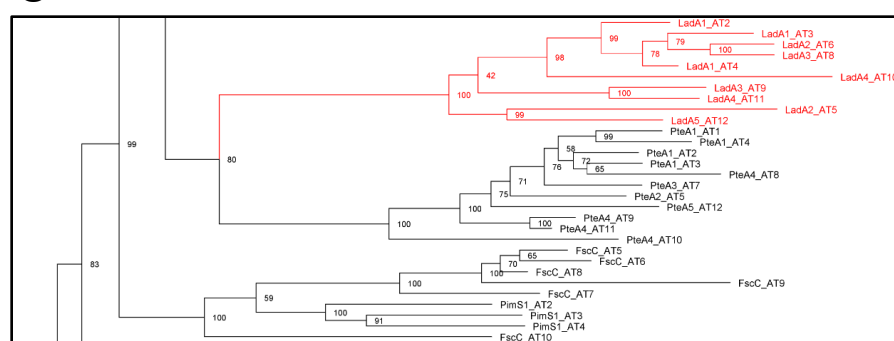
A



B



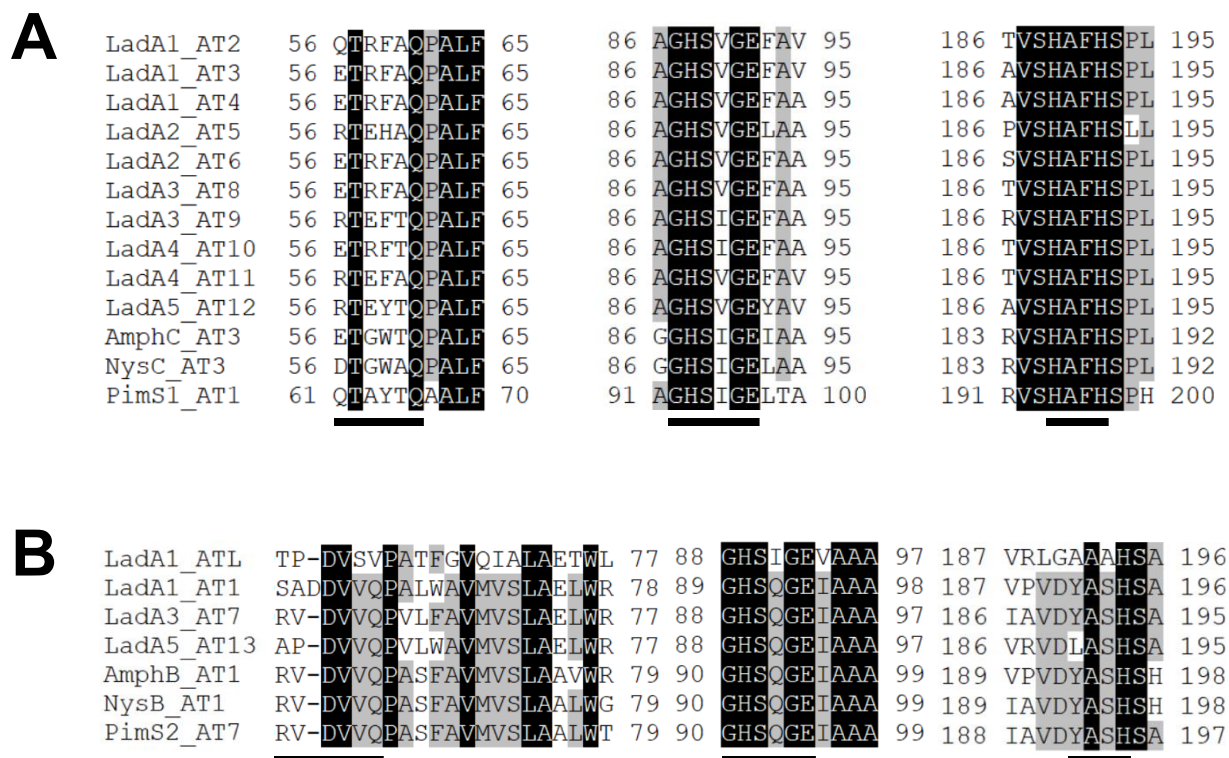
C



0.3

## Figure S4

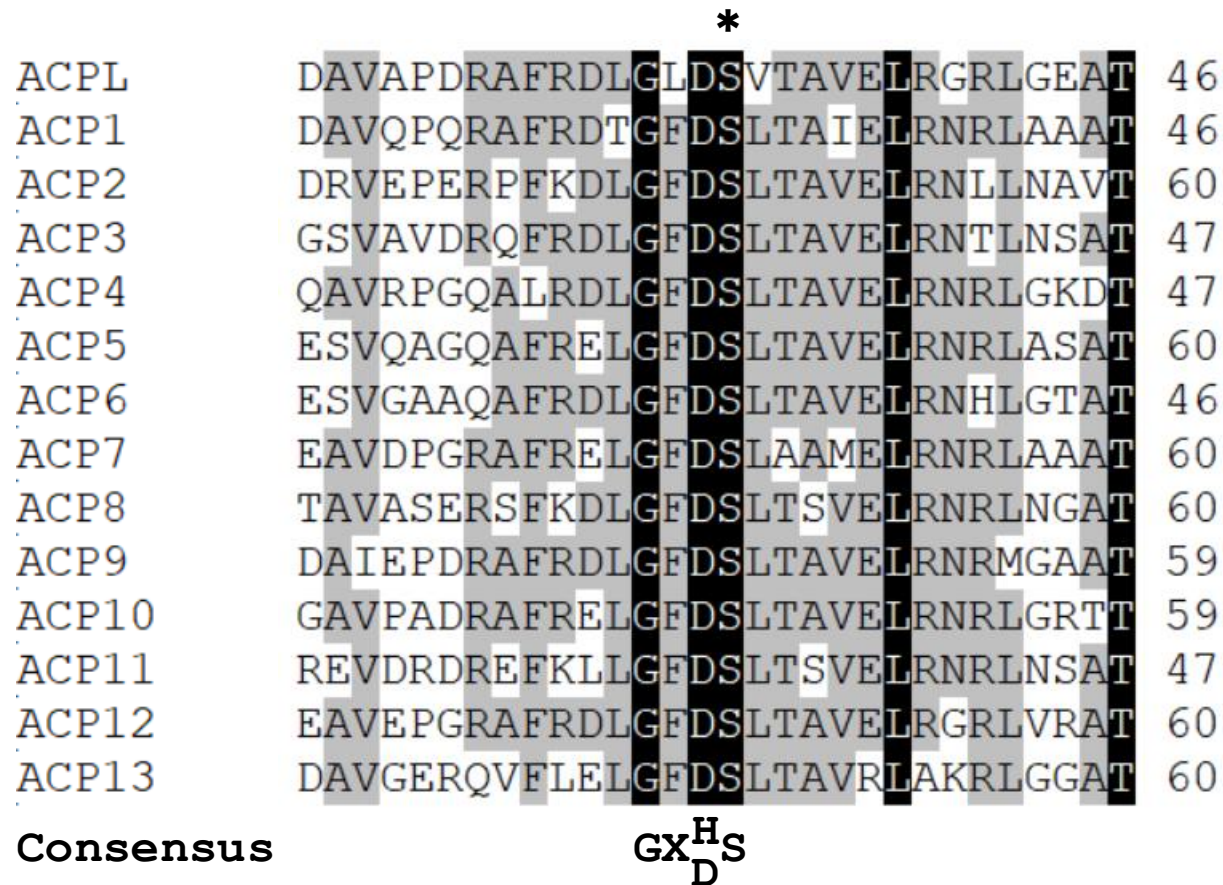
Analysis of the AT domains from the LadA PKs using a phylogenetic tree. Posterior probabilities are shown at the nodes, and the bar indicates substitutions per site. Subclades colored red indicate AT domains on the lavencidin biosynthesis gene cluster. Amino acid sequences of AT domains were retrieved from the databases DoBISCUIT (<https://www.nite.go.jp/nbr/pks/>) and ClusterCAD (<https://clustercad.jbei.org/>). These amino acid sequences were aligned with Clustal Omega (1.2.2) implemented in Geneious Prime 2023.2.1 (Biomatters Ltd.) with automatically adjusted settings for the number of sequences in refinement iterations. Bayesian phylogeny was conducted by MrBayes (ver. 3.2.7a) on CIPRES Science Gateway (<http://www.phylo.org/>) with the following parameters: aamodelpr = fixed(LG); ngen = 20 000 000; rateopts = invgamma; nrns = 2; nchains = 4; temp = 0.2; relburnin = yes; burninfrac = 0.25. The substitution model was selected by ModelTest-NG. The phylogenetic tree was rendered with FigTree version 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). (A) Overall diagram of the phylogenetic tree of AT domains. (B) Partial diagram of the phylogenetic tree of AT domains including LadA1\_ATL, LadA1\_AT1, LadA3\_AT7, and LadA5\_AT13. (C) Partial diagram of the phylogenetic tree of AT domains including LadA1\_AT2, LadA1\_AT3, LadA1\_AT4, LadA2\_AT5, LadA2\_AT6, LadA3\_AT8, LadA3\_AT9, LadA4\_AT10, LadA4\_AT11, and LadA5\_AT12.



**Figure S5**

Analysis of the AT domains from LadA PKs by amino acid sequence alignment. The numbers indicate amino acid positions within each domain. Black and gray boxes in the alignment indicate positions at which the same amino acid is found and at least 70% of the amino acids match, respectively. The AT domains and their corresponding sequence accession numbers are as follows: AmphB/AmphC (AAK73513/AAK73514) for amphotericin in *Streptomyces nodosus* ATCC 14899; NysB/NysC (AVX51107/AVX51108) for nystatin in *Streptomyces noursei* ATCC 11455; PimS1/PimS2 (CAC20931/CAC20921) for pimarinin in *Streptomyces natalensis* ATCC 27448. Three dominant motifs, composed of six, six, and four amino acid residues, are underlined. (A) Sequence alignment of the conserved motifs in AT domains including LadA1\_AT2, LadA1\_AT3, LadA1\_AT4, LadA2\_AT5, LadA2\_AT6, LadA3\_AT8, LadA3\_AT9, LadA4\_AT10, LadA4\_AT11, and LadA5\_AT12. (B) Sequence alignment of the conserved motifs in AT domains including LadA1\_ATL, LadA1\_AT1, LadA3\_AT7, and LadA5\_AT13.

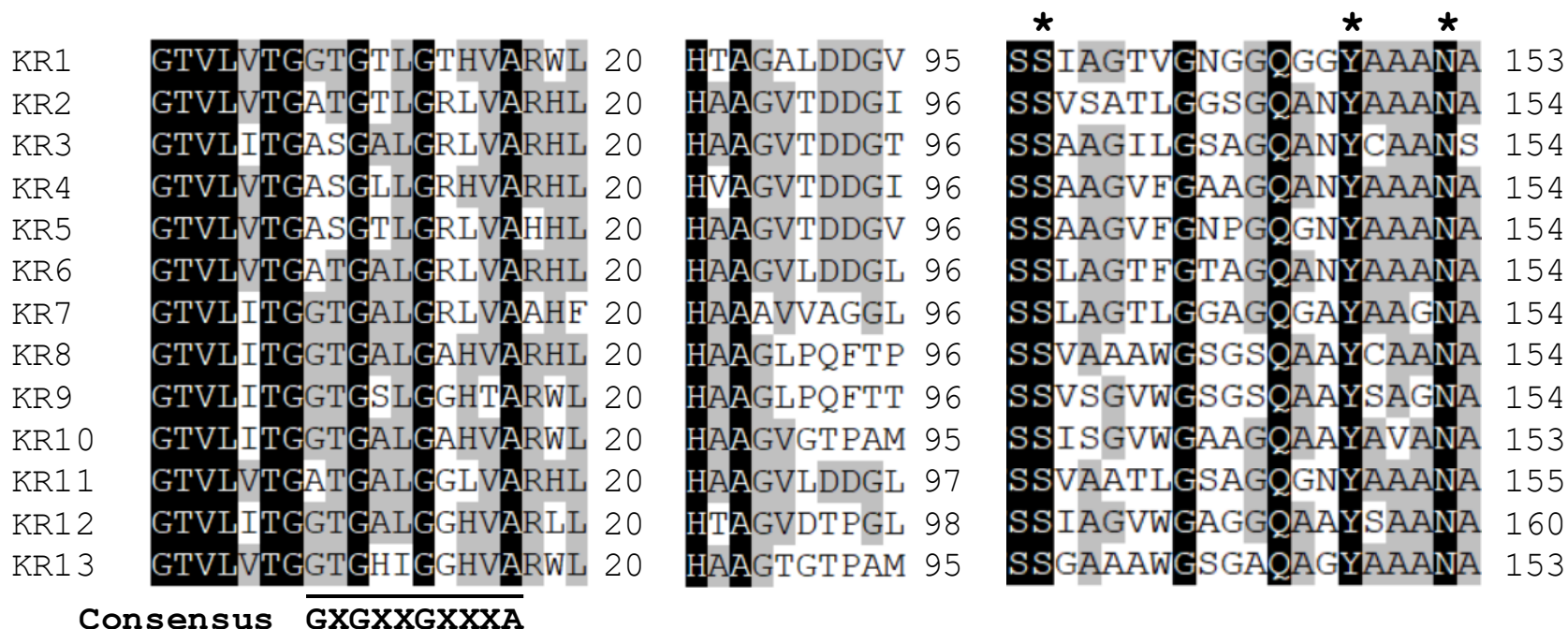




**Figure S6**

Sequence alignment of the conserved motifs in the ACP domain core regions from LadA PKSs. The numbers indicate amino acid positions within each domain. Black and gray boxes in the alignment indicate positions at which the same amino acid is found and at least 70% of the amino acids match, respectively. An asterisk indicates the active site Ser residue acting as the phosphopantetheine-binding site.





**Figure S7**

Sequence alignment of the conserved motifs in the KR domain core regions from LadA PKSs. The numbers indicate amino acid positions within each domain. Black and gray boxes in the alignment indicate positions at which the same amino acid is found and at least 70% of the amino acids match, respectively. The consensus sequence for the NADP(H)-binding site is underlined. The catalytic triad of S-Y-N is indicated by asterisks.

	*		*	*
DH2	WIADHTVSGAVLVPGTAFVE	58	ALLDAALHASWLG--	218
DH3	WLADHAVSGVTLLPGTAFE	59	ALLDAALHAMSVGEL	226
DH4	WLGDHTVMDAILLPGTALVD	59	ALLDSALHAIGLGGL	222
DH5	WLAGHRVLGSPLLPGTALVE	59	ALFDAALHTAGLGG-	220
DH6	WLADHVVLGAVLVPGTAFVD	59	ALLDAALHAVALGDG	225
DH7	WLAEHEVLGAVILPGTAFVE	59	ALLDAALHAVGFGP-	223
DH11	WLAQHQVMGAALLPGTALVD	59	ALLDSALHAVGLGGL	229
<b>Consensus</b>	HXXXGXXXXP			

### Figure S8

Sequence alignment of the conserved motifs in the DH domain core regions from LadA PKSs. The numbers indicate amino acid positions within each domain. Black and gray boxes in the alignment indicate positions at which the same amino acid is found and at least 70% of the amino acids match, respectively. The conserved HXXGXGXXXP motif for DH domains is underlined. The proposed catalytic His, Asp, and His residues are marked with asterisks.

ER11      LLVHSAAGGVGMAA      144

**Consensus**      **LXHXXXGGVG**

**Figure S9**

Sequence alignment of the conserved motif in the ER11 domain core region from LadA4 PKS. The number indicates the amino acid position within the domain. The conserved amino acids in the proposed NADP(H)-binding site LXHXXXGGVG are highlighted in gray.