Table S1. Oligonucleotides used in this study

Primer	Sequence (5' – 3')
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
PmCDA1$_{str}$	ATGACCGACGCCGAGTACGTCCGCATCCACGAGAAGCTGGACATCTACA CCTTCAAGAAGCAGTTCTTCAACAACAAGAAGTCCGTCAGCCACCGCTG CTACGTCCTGTTCGAGCTGAAGCGCCGGGGCGAGCGCCGCGCCTGCTTC TGGGGCTACGCCGTCAACAAGCCGCAGTCCGGCACCGAGCGGGGCATCC ACGCCGAGATCTTCTCCATCCGCAAGGTCGAGGAGTACCTGCGCGACAA CCCGGGCCAGTTCACGATCAACTGGTACTCCAGCTGGTCCCCGTGCGCC GACTGCGCCGAGAAGATCCTCGAGTGGTACAACCAGGAGCTGCGCGGCA ACGGCCACACGCTGAAGATCTGGGCCTGCAAGCTGTACTACGAGAAGAA CGCCCGCAACCAGATCGGCCTGTGGAACCTCCGCGACAACGGCGTCGGC CTGAACGTCATGGTGTCCGAGCACTACCAGTGCTGCCGCAAGATCTTCA TCCAGTCCTCGCACAACCAGCTGAACGAGAACCGCTGGCTCGAGAAGAC CCTGAAGCGGGCCGAGAAGCGCCGCTCCGAGCTGTCCATCATGATCCAG GTCAAGATCCTGCACACCACCAAGTCGCCGGCCGTC
UGI$_{str}$	ATGACCAACCTGTCCGACATCATCGAGAAGGAGACCGGCAAGCAGCTGG TCATCCAGGAGTCCATCCTGATGCTCCCCGAGGAGGTCGAGGAGGTCAT CGGCAACAAGCCCGAGTCCGACATCCTGGTCCACACCGCCTACGACGAG TCCACCGACGAGAACGTGATGCTGCTGACCTCCGACGCGCCCGAGTACA AGCCGTGGGCGCTCGTGATCCAGGACTCCAACGGCGAGAACAAGATCAA GATGCTG
LVA$_{str}$	CTCGTCGCC
Linker region (GS linker , SH3 domain and 3×FLAG tag)	GGCGGCGGCGGCTCCGGCGGCGGCGGCAGCGCCGAGTACGTGCGCGCCC TGTTCGACTTCAACGGCAACGACGAGGAGGACCTGCCGTTCAAGAAGGG CGACATCCTGCGCATCCGCGACAAGCCCGAGGAGCAGTGGTGGAACGCC GAGGACTCCGAGGGCAAGCGCGGCATGATCCCGGTGCCGTACGTCGAGA AGTACTCCGGCGACTACAAGGACCACGACGGGGACTACAAGGACCACGA CATCGACTACAAGGACGACGACGACAAGTCCCGCCTCGAGTCGGGCGAC TACAAGGACCACGACGGGGACTACAAGGACCACGACATCGACTACAAGG ACGACGACGACAAGAGCCGC

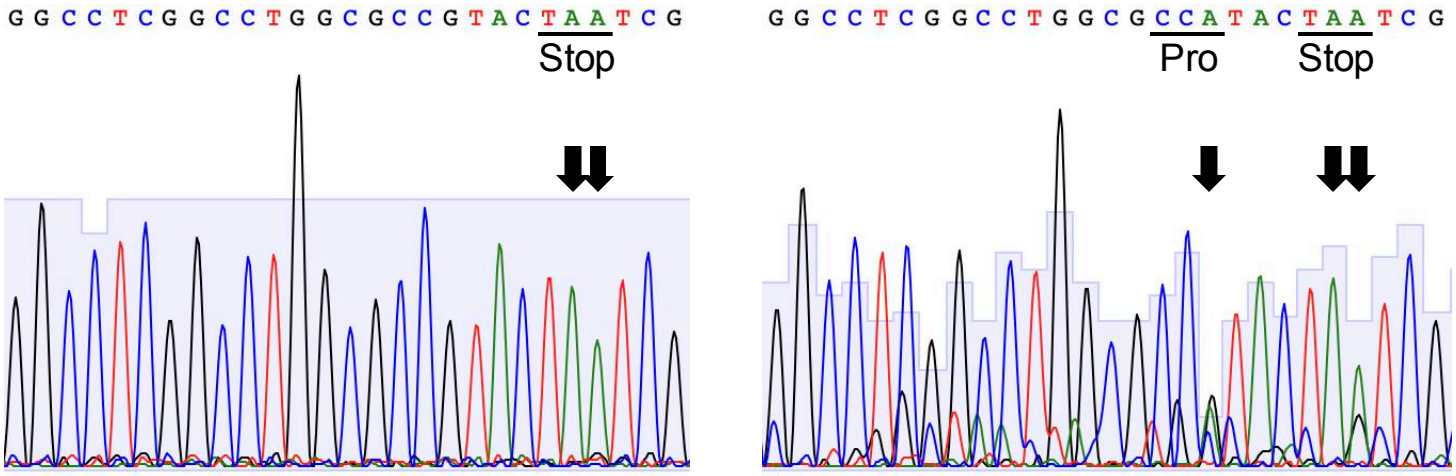

Figure S1
Sanger sequencing chromatograms of the target region with the predicted C to T mutation in the m*actI-ORF2* strains. Mutated bases are indicated by black arrows.

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.

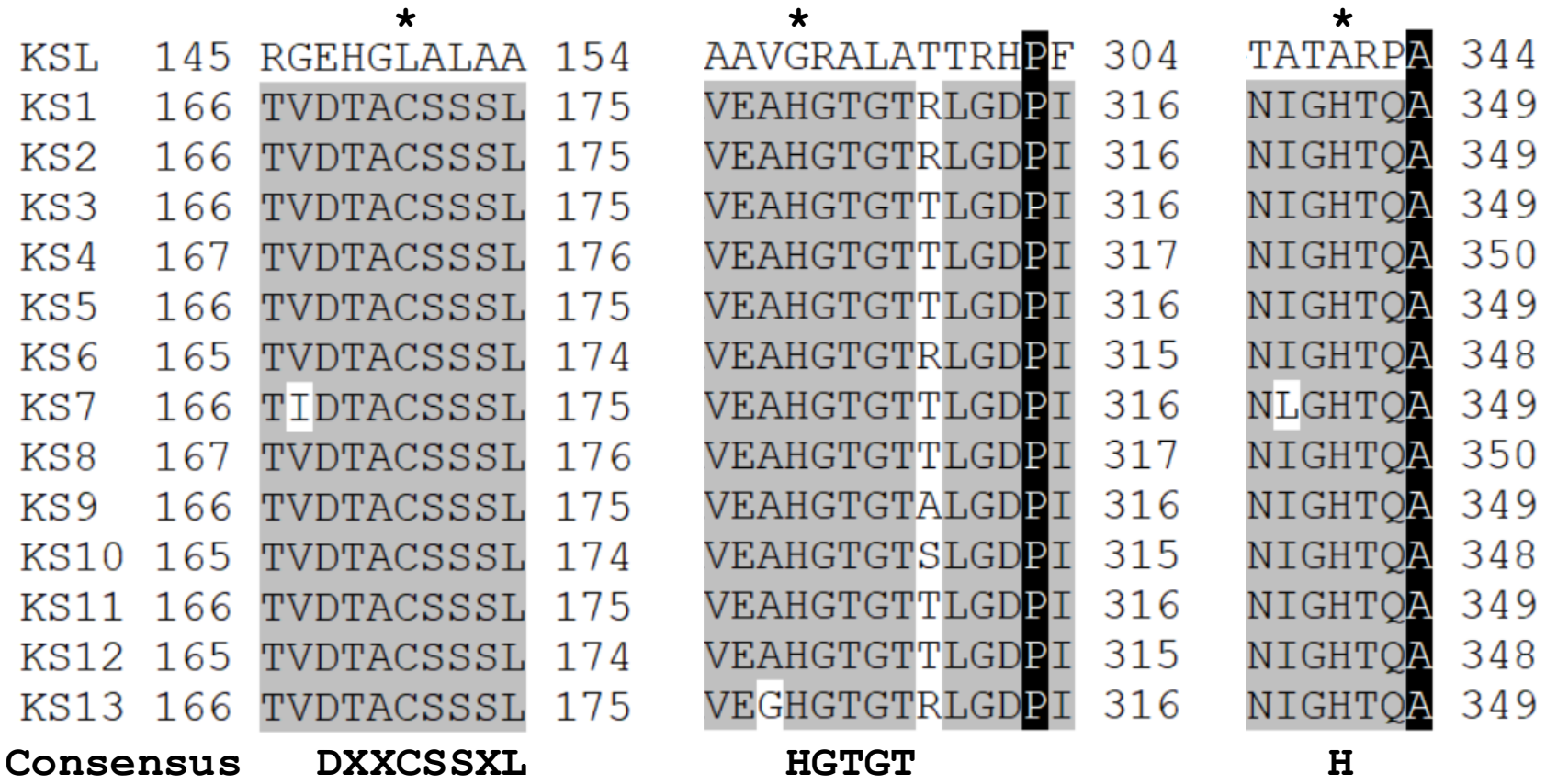

Figure S3

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.

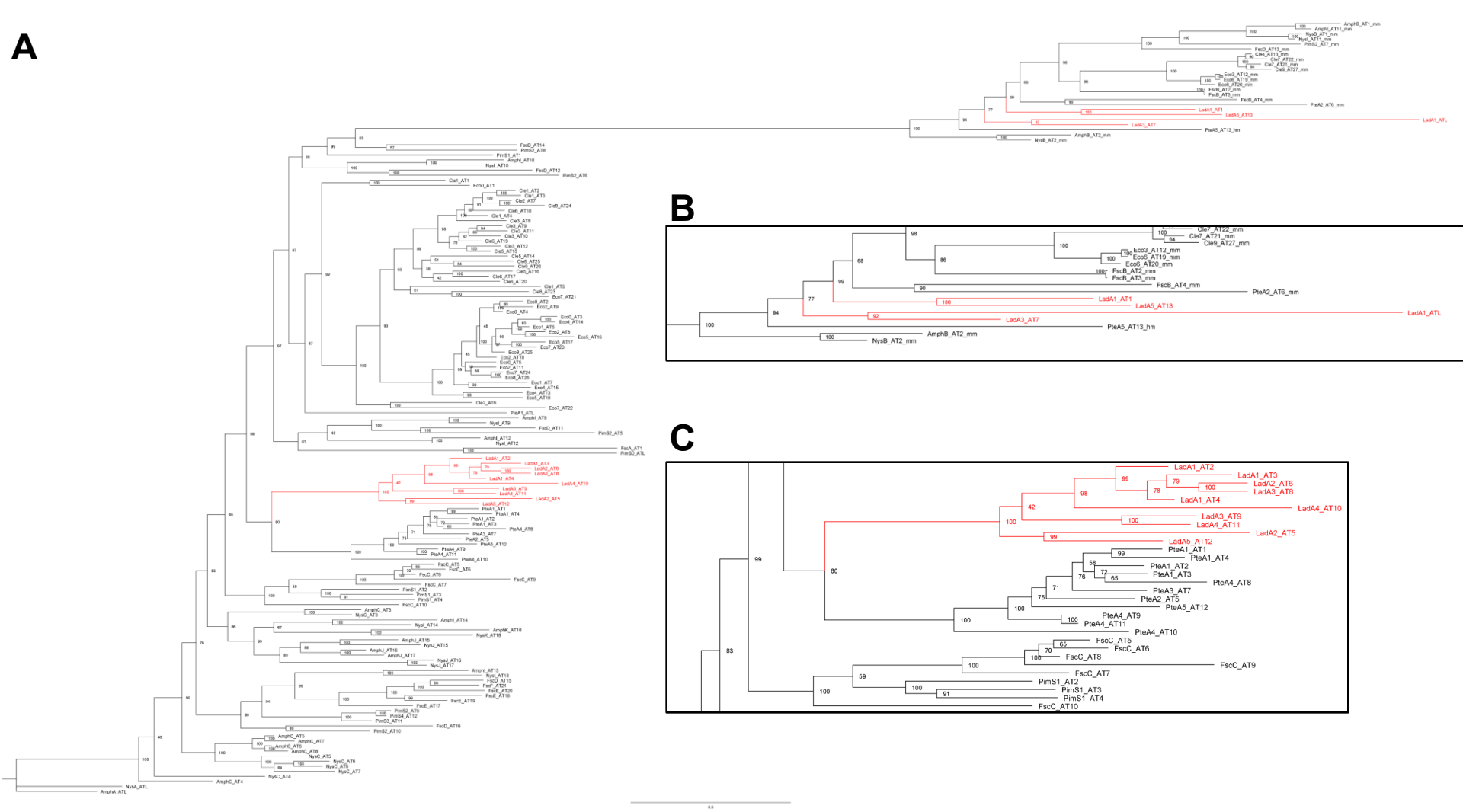

Figure S4

Analysis of the AT domains from the LadA PKSs 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/nbrc/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; nruns = 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.

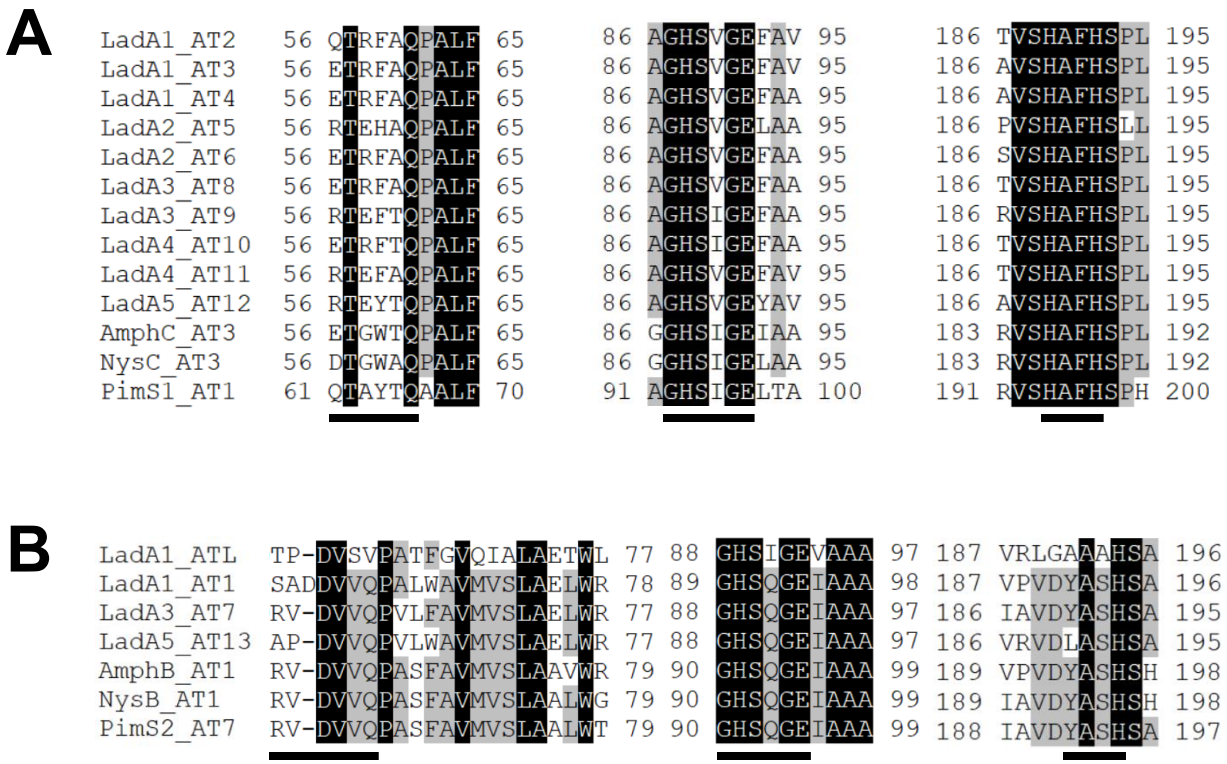

Figure S5

Analysis of the AT domains from LadA PKSs 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 pimaricin 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.

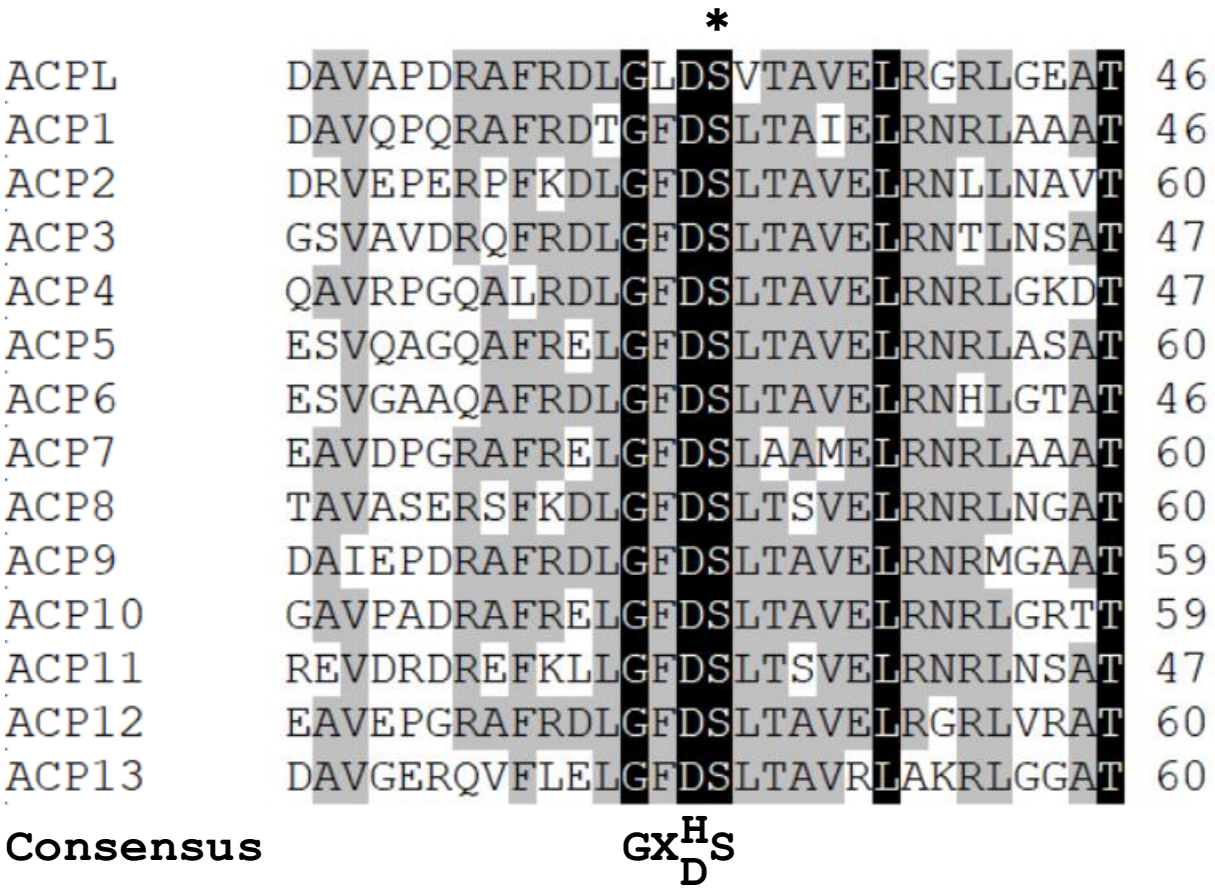

Figure S6

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.

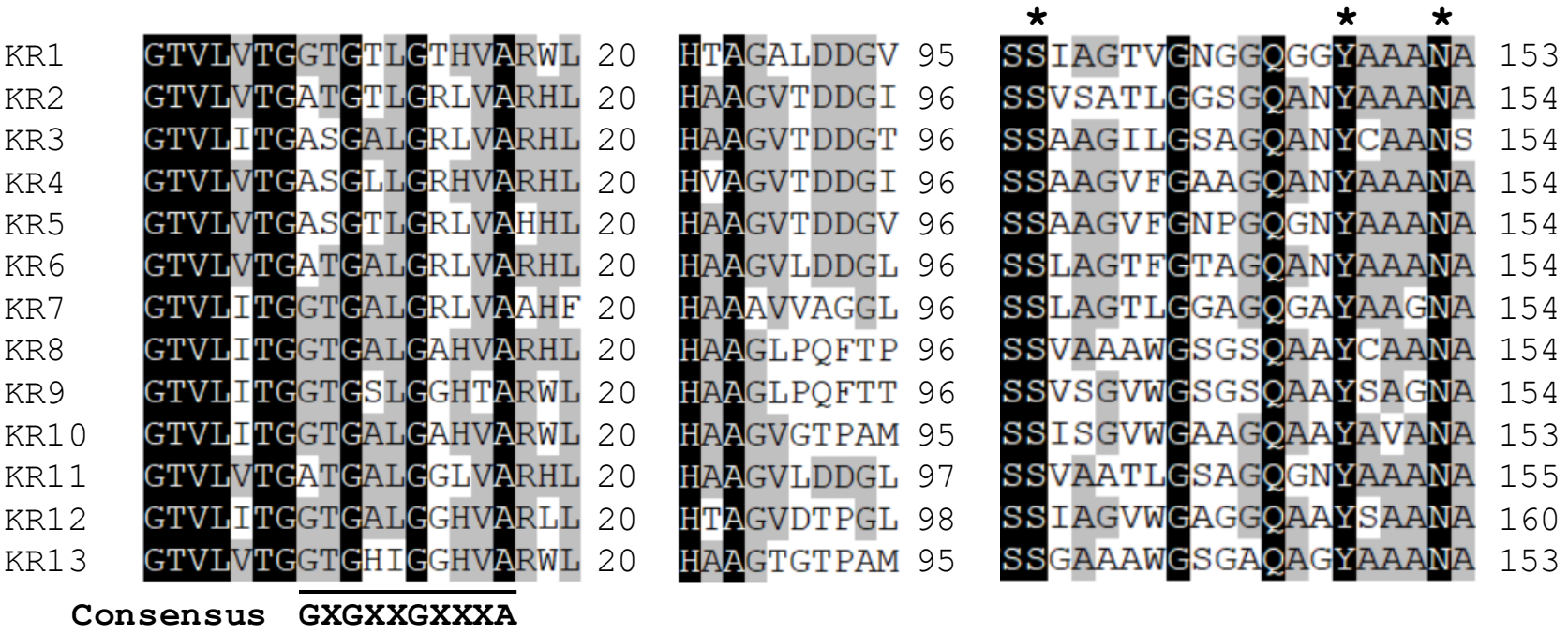

Figure S7

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.

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 HXXXGXXXXP motif for DH domains is underlined. The proposed catalytic His, Asp, and His residues are marked with asterisks.

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.