The Discovery of Weddellamycin, a Tricyclic Polyene Macrolactam Antibiotic from an Antarctic Deep-Sea-Derived *Streptomyces* sp. DSS69, by Heterologous Expression

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Table S1. antiSMASH-predicted BGCs for *Streptomyces* sp. *DSS69.*

BGCs	Position		Type	Most similar known cluster/Similarity
	from	to		
Cluster 1	115,769	126,656	butyrolactone	coelimycin P1 / 16%
Cluster 2	155,631	177,513	terpene	geosmin / 100%
Cluster 3	217,831	270,224	NRPS	griseobactin / 100%
Cluster 4	294,564	350,623	NRPS	coelichelin / 81%
Cluster 5	360,973	399,099	T3PKS	lasalocid / 9%
Cluster 6	680,259	722,829	NRPS	diisonitrile antibiotic SF2768 / 66%
Cluster 7	1,020,062	1,039,917	terpene	steffimycin D / 19%
Cluster 8	1,485,361	1,495,259	ectoine	ectoine / 100%
Cluster 9	2,073,506	2,146,039	T2PKS-oligosaccharide	chromomycin A3 / 100%
Cluster 10	2,438,892	2,469,166	lanthipeptide	—
Cluster 11	2,524,339	2,534,794	siderophore	desferrioxamin B / 100%
Cluster 12	2,883,003	2,916,534	LAP-thiopeptide	—
Cluster 13	3,034,928	3,109,440	NRPS-PKS-like	omnipeptin / 7%
Cluster 14	3,136,407	3,188,955	NRPS	phosphonoglycans / 3%
Cluster 15	3,311,291	3,417,260	T1PKS	bombyxamycin A / 50%
Cluster 16	3,426,050	3,479,268	lanthipeptide-NRPS	albomycin delta2 / 10%
Cluster 17	3,669,942	3,697,519	betalactone	divergolide / 6%
Cluster 18	4,115,741	4,136,103	nucleoside	toyocamycin / 30%
Cluster 19	4,305,025	4,327,695	lassopeptide	keywimysin / 100%
Cluster 20	5,107,055	5,129,670	lanthipeptide	AmfS / 100%
Cluster 21	5,454,333	5,473,242	terpene	—
Cluster 22	5,846,508	5,861,249	siderophore	kanamycin / 20%
Cluster 23	6,176,336	6,186,306	RiPP-like	—
Cluster 24	6,327,416	6,373,542	NRPS	salinomycin / 14%
Cluster 25	6,426,691	6,477,940	ectoine	kosinostatin / 13%
Cluster 26	6,786,620	6,812,714	terpene	hopene / 69%
Cluster 27	6,864,977	6,914,966	NRPS	a201a / 8%
Cluster 28	6,927,927	6,976,192	TIPKS-NRPS	10-epi-HSAF / 100%
Cluster 29	6,991,610	6,999,604	RiPP-like	tetronasin / 3%
Cluster 30	7,091,171	7,144,941	NRPS	thiocoraline / 21%
Cluster 31	7,222,132	7,231,365	RiPP-like	streptamidine / 66%
Cluster 32	7,233,826	7,244,293	melanin	istamycin / 4%
Cluster 33	7,315,596	7,356,648	T3PKS	alkylresorcinol / 100%
Cluster 34	7,364,029	7,430,038	NRPS	crochelin A / 12%
Cluster 35	7,514,850	7,540,412	terpene	isorenieratene / 100%
Cluster 36	7,554,728	7,606,949	T1PKS-NRPS-thiopeptide	lactazole / 33%

Table S2. Predicted function of the genes from the weddellamycin BGC.

No.	Gene	Size [bp](aa)	Putative function	Homolog	Identity [%] / Similarity [%]
1	*orf1*	1533 (510)	alpha / beta hydrolase fold protein	(AAP03104.1)	69 / 92
2	*orf2*	789 (262)	thioesterase	CmiR1 (BAO66517.1)	71 / 98
3	*orf3*	1128 (375)	putative transcriptional regulator	BomR5 (QBL56177.1)	61 / 55
4	*wdlA*	2745 (914)	LuxR family transcriptional regulator	BomR4 (QBL56174.1)	62 / 100
5	*wdlB*	603 (200)	TetR family transcriptional regulator	MlaM (ACO94493.1)	50 / 93
6	*wdlC*	270 (89)	unknown	(QBL56203.1)	74 / 100
7	*wdlD*	759 (252)	type II thioesterase	BomR9 (QBL56202.1)	72 / 97
8	*wdlE*	948 (315)	ACP S-malonyltransferase	BomM (QBL56199.1)	73 / 100
9	*wdlF*	870 (289)	sugar phosphate isomerase / epimerase	BomL (QBL56198.1)	66 / 99
10	*wdlG*	1233 (410)	cytochrome P450	BomK (QBL56197.1)	88 / 100
11	*wdlH*	195 (4)	ferredoxin	(QBL56196.1)	88 / 100
12	*wdlI*	1659 (552)	long chain fatty acid CoA ligase	BomJ (QBL56195.1)	78 / 92
13	*wdlJ*	1260 (419)	diaminopimelate decarboxylase	BomI (QBL56194.1)	81 / 99
14	*wdlK*	501 (166)	glutamate mutase sigma subunit 2	BomH (QBL56193.1)	73 / 89
15	*wdlL*	1428 (475)	methylaspartate mutase	BomG (QBL56192.1)	75 / 89
16	*wdlM1*	16398 (5465)	PKS		
17	*wdlM2*	5322 (1773)	PKS		
18	*wdlN*	1206 (401)	FAD-dependent oxidoreductase	BomF (QBL56190.1)	74 / 99
19	*wdlO*	756 (251)	TetR family transcriptional regulator	BomR8 (QBL56189.1)	80 / 100
20	*wdlP*	1656 (551)	transporter	(QBL56188.1)	81 / 94
21	*wdlQ*	1602 (533)	long chain fatty acid CoA ligase	BomE (QBL56187.1)	73 / 99
22	*wdlR*	1035 (344)	transposase	(QBL56186.1)	54 / 91

(table to be continued)

(table continued)					
23	*wdlS*	237 (78)	acyl carrier protein	BomD (QBL56185.1)	76 / 100
24	*wdlM3*	10644 (3547)	PKS		
25	*wdlM4*	5019 (1672)	PKS		
26	*wdlM5*	10995 (3664)	PKS		
27	*wdlM6*	11376 (3788)	PKS		
28	*wdlT*	909 (302)	L-amino acid amidase	BomC (QBL56180.1)	87 / 99
29	*wdlU*	405 (134)	GntR family transcriptional regulator	(MXG30154.1)	99 / 99
30	*wdlV*	975 (324)	ABC transporter permease subunit	(WP_109164968.1)	100 / 100
31	*wdlW*	945 (314)	putative ABC transporter ATP-binding protein	(ACB47083.1)	44 / 89
32	*orf4*	684 (227)	phosphotransferase	(WP_109164969.1)	99 / 99

Table S3. Primers used in this study.

Primer	Sequence (5′→3′)	Uses
15-1-F	CGGGGAGGGGGAGTCAGATG	Primers for BAC plasmid screening
15-1-R	CAGGGCAACTTCTGGGCTCG	
15-2-F	CGTTCGCTGCGGGAGGTCATC	
15-2-R	GCTTCCAGGGTGAGTTCCTC	
15-3-F	GTGCAGATGACTGAGTCGGG	
15-3-R	GCCGCTCCAGGACGAAGACG	
15-4-F	TCAGGTGCACTTCTTGTCGT	
15-4-R	GCTATCTCCAGGGGTACGCG	
*Δwdl*A-F	TTGACTTCTTTGCATGTCCTTGACTGCTGTGGGCGGTC ATGTAGGCTGGAGCTGCTTC	Primers for disrupting *wdlA*
*Δwdl*A-R	CGGAAGCAGGCCGGTCAGCAGGATCTGTCCAGTGA AGTGATTCCGGGGATCCGTCGACC	
*Δwdl*A-YZ-F	TACTGTCAGTGGCGAAACGG	Primers for *ΔwdlA* construct verification
*Δwdl*A-YZ-R	CCGGCCCACCGTACGTGAGT	
*Δwdl*B-F	ACCCGGCGGGGACCACCCGCACCACGGTCCCGGCC GTCATGTAGGCTGGAGCTGCTTC	Primers for disrupting *wdlB*
*Δwdl*B-R	GATGGCCGCCACGACCGCGAGACGAGGGGGTGTGC CGTGATTCCGGGGATCCGTCGACC	
*Δwdl*B-YZ-F	GGTCACTAGTTGCACCGTGC	Primers for *ΔwdlB* construct verification
*Δwdl*B-YZ-R	GCCCCCGAGAACACCCGTAG	
*Δwdl*F-F	GGAGTTCGGTGGCGGTGAGGGGGGCTCGGTGAGGG CCTATGTAGGCTGGAGCTGCTTC	Primers for disrupting *wdlF*
*Δwdl*F-R	GGTCGGCCGTGAACTGTCGAAGGCGAGAGGTACCCC ATGATTCCGGGGATCCGTCGACC	
*Δwdl*F-YZ-F	GTTCGGTGGCGGTGAGGGGG	Primers for *ΔwdlF* construct verification
*Δwdl*F-YZ-R	GGTCGGCCGTGAACTGTCGA	
*Δwdl*G-F	CGACGATCACACGCATGGAACCGCTCCTTTACCAGG TCATGTAGGCTGGAGCTGCTTC	Primers for disrupting *wdlG*
*Δwdl*G-R	CACCGAACTCCGCCAAAGAGCTGAAGAGAGGCCAC CATGATTCCGGGGATCCGTCGACC	
*Δwdl*G-YZ-F	ACGATCACACGCATGGAACC	Primers for *ΔwdlG* construct verification
*Δwdl*G-YZ-R	CGCCAAAGAGCTGAAGAGAG	
*Δwdl*H-F	CGCCCGGTCGGTGGTCCGGGTGCGCGGGGGGCCGCG CTATGTAGGCTGGAGCTGCTTC	Primers for disrupting *wdlH*
*Δwdl*H-R	CACGAACTCCCGGTGACCTGGTAAAGGAGCGGTTCC ATGATTCCGGGGATCCGTCGACC	
*Δwdl*H-YZ-F	CGGTCGGTGGTCCGGGTG	Primers for *ΔwdlH* construct verification
*Δwdl*H-YZ-R	GACCTGGTAAAGGAGCGGTT	
*Δwdl*O-F	TCCGGGGCGGGGGACCAGCCCCCTTCGGCCTGTGTG TCATGTAGGCTGGAGCTGCTTC	Primers for disrupting *wdlO*
*Δwdl*O-R	TACGCACGGCCCTGCATCCACGAAGGTGAGCACCTT ATGATTCCGGGGATCCGTCGACC	
*Δwdl*O-YZ-F	CTGCCGGCAGTTCCTCGGTG	Primers for *ΔwdlO* construct verification
*Δwdl*O-YZ-R	CTCGGCGACTACGAGACCAC	

(table to be continued)

(table continued)

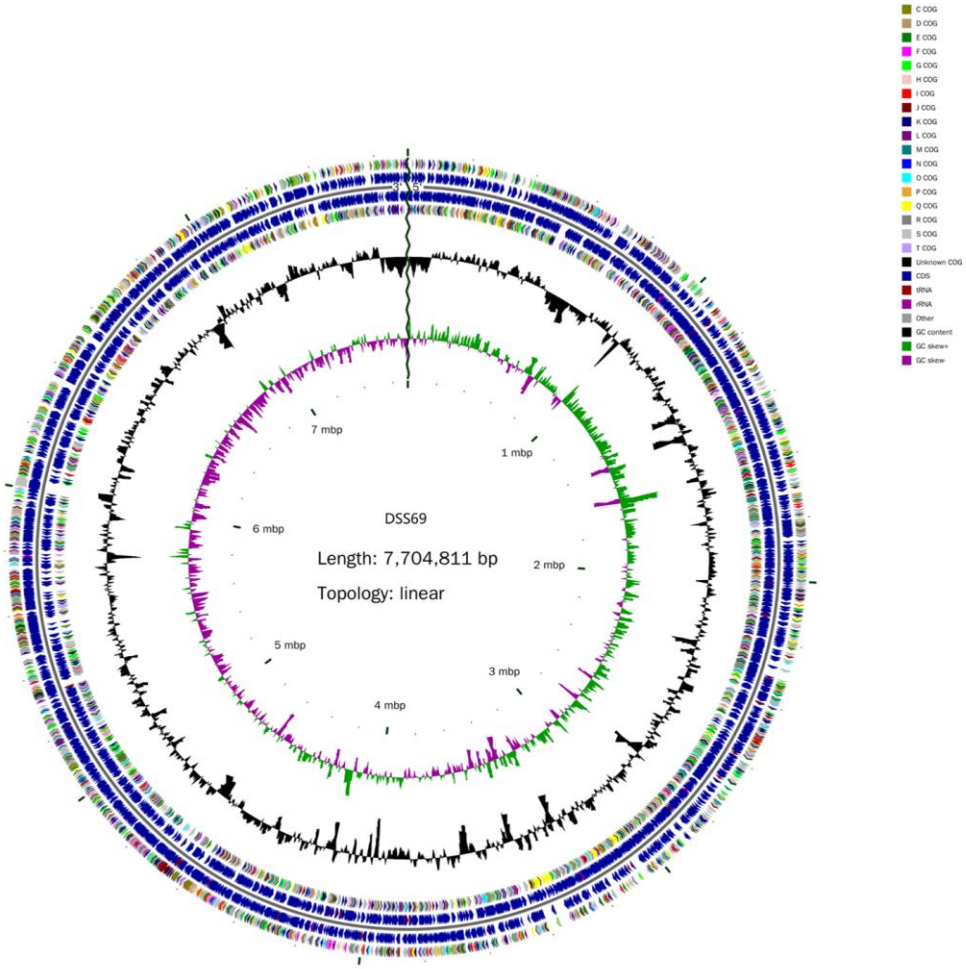
*Δwdl*U-F	CTGACCCCGCGACCCACCCACGTACGGTCGGGTCCG TCATGTAGGCTGGAGCTGCTTC	Primers for disrupting *wdlU*
*Δwdl*U-R	CGTAAAGTGTCGGCAATCGAGCGGAAGGTGAAGTC CGTGATTCCGGGGATCCGTCGACC	
*Δwdl*U-YZ-F	GTGGGAGATCTTCGAGGAGT	Primers for *ΔwdlU* construct verification
*Δwdl*U-YZ-R	AGCACGGGATCATCAACGAC	
kasOp*-wdlA-ter-F	AATTCGATATCGCGCGCGGCCGCtgttcacattcgaacggtctct gctttgacaacatgctgtgcggtgttgtaaagtcgtggccaggagaatacgacagc gtgcaggactgggggagttCATATGGTGCCGGGGGACAAAGT GGT (*Not*I, *Nde*I)	Primers for pCL08 construction
kasOp*-wdlA-ter-R	TCGTTAGTTAGGCTAACTAGTaaaaaaaaccccgccctgtcaggg gcggggttttttttttctctagtaGGCGGTCAGGCCACGTCCATGGC CG (*Spe*I)	
pCL08-YZ-1-F	GACAACATGCTGTGCGGTGT	Primers for pCL08 construct verification
pCL08-YZ-1-R	GGGCTCCGGTCGAGTACGTC	
kasOp*-wdlB-ter-1-F	AATTCGATATCGCGCGCGGCCGCtgttcacattcgaacggtctct gctttgacaacatgctgtgcggtgttgtaaagtcgtggccaggagaatacgacagc gtgcaggactgggggagttCATATGGTGGGTCACCGTGAGGA CTT (*Not*I, *Nde*I)	Primers for pCL09 construction
kasOp*-wdlB-ter-1-R	TCGTTAGTTAGGCTAACTAGTaaaaaaaaccccgccctgtcaggg gcggggttttttttttctctagtaGGCCGTCACTTCACGACGGCCTC GT (*Spe*I)	
pCL09-YZ-F	CGATATCGCGCGCGGCCGCT	Primers for pCL09 construct verification
pCL09-YZ-R	CGTTAGTTAGGCTAACTAGT	
kasOp*-wdlB-ter-2-F	ACCATGCATAGATCTAAGCTTaaaaaaaaccccgccctgtcagg ggcggggttttttttttctctagtaGGCCGTCACTTCACGACGGCCT CGT (*Hin*dIII)	Primers for pCL10 construction
kasOp*-wdlB-ter-2-R	GCGAAAAGCCGAGAACCTAGGtgttcacattcgaacggtctctgc tttgacaacatgctgtgcggtgttgtaaagtcgtggccaggagaatacgacagcgt gcaggactgggggagttCATATGGTGGGTCACCGTGAGGAC TT (*Avr*II, *Nde*I)	
pCL10-YZ-F	ACCATGCATAGATCTAAGCT	Primers for pCL10 construct verification
pCL10-YZ-R	ATGGCGAAAAGCCGAGAACC	

Table S4. Strains and plasmids used and constructed in this study.

Strain or Plasmid	Description	Sources or References
Streptomyces		
S. lividans GX28	Host for heterologous expression of BACs	[1]
S. lividans GX28/vector	*S. lividans* GX28 contains empty vector pMSBBAC1	This work
S. lividans GX28 /pBAC-wdl	*S. lividans* GX28 integrated with plasmid pBAC-wdl which contains *wdl* biosynthetic gene cluster	This work
ΔwdlA	*wdlA* inactivation mutant of *S. lividans* GX28/pBAC-wdl	This work
ΔwdlB	*wdlB* inactivation mutant of *S. lividans* GX28/pBAC-wdl	This work
ΔwdlF	*wdlF* inactivation mutant of *S. lividans* GX28/pBAC-wdl	This work
ΔwdlG	*wdlG* inactivation mutant of *S. lividans* GX28/pBAC-wdl	This work
ΔwdlH	*wdlH* inactivation mutant of *S. lividans* GX28/pBAC-wdl	This work
ΔwdlO	*wdlO* inactivation mutant of *S. lividans* GX28/pBAC-wdl	This work
ΔwdlU	*wdlU* inactivation mutant of *S. lividans* GX28/pBAC-wdl	This work
OwdlA	Plasmid pCL05 was integrated into the starin *S. lividans* GX28/pBAC-wdl for overexpressing gene *wdlA*	This work
OwdlB	Plasmid pCL06 was integrated into the starin *S. lividans* GX28/pBAC-wdl for overexpressing gene *wdlB*	This work
OwdlAB	Plasmid pCL07 was integrated into the starin *S. lividans* GX28/pBAC-wdl for overexpressing gene *wdlA* and gene *wdlB*	This work
ΔwdlO+OwdlA	Plasmid pCL05 was integrated into the starin *S. lividans* GX28/*ΔwdlO* for overexpressing gene *wdlA*	This work
ΔwdlO+OwdlB	Plasmid pCL06 was integrated into the starin *S. lividans* GX28/*ΔwdlO* for overexpressing gene *wdlB*	This work
ΔwdlO+OwdlAB	Plasmid pCL07 was integrated into the starin *S. lividans* GX28/*ΔwdlO* for overexpressing gene *wdlA* and gene *wdlB*	This work
E. Coli		
DH10B	Host strain for cloning	Invitrogen
BW25113/pIJ790	Host strain for λ Red-mediated PCR targeting	[2]
DH5α/BT340	Host strain for in-frame deletion	[2]
ET12567	Host strain of BACs	[3]
ET12567/pUB307	Helper strain for conjugation between *E. coli* and *Streptomyces*	[4]
Plasmids		(table to be continued)

(table continued)

pMS82	*Hyg*^{r}, *oriT*, *int-attP*$^{\varphi BT1}$, integrative plasmid	[5]
pJTU6722	*Ery*^{r}, PCR template for *eryB* cassette	[6]
pMSBBAC1	*oriT*, *int-attP*$^{\varphi C31}$, *Apr*^{r}, BAC vector	[7]
pBAC-wdl	BAC contains the weddellamycin biosynthetic gene cluster	This work
pCL01	*wdlA* was deleted from pBAC-wdl	This work
pCL02	*wdlB* was deleted from pBAC-wdl	This work
pCL03	*wdlF* was deleted from pBAC-wdl	This work
pCL04	*wdlG* was deleted from pBAC-wdl	This work
pCL05	*wdlH* was deleted from pBAC-wdl	This work
pCL06	*wdlO* was deleted from pBAC-wdl	This work
pCL07	*wdlU* was deleted from pBAC-wdl	This work
pCL08	pMS83 was inserted into a *kasO*p*-*wdlA-ter* cassette	This work
pCL09	pMS83 was inserted into a *kasO*p*-*wdlB-ter-1* cassette	This work
pCL10	pMS83 was inserted into a *kasO*p*-*wdlA-ter* cassette and a *kasO*p*-*wdlB-ter-2* cassette	This work



Feature	Chromosome Characteristics
Genome topology	Linear
Chromosome size (bp)	7,704,811
Average GC content (%)	71.61%
Protein-coding genes	6,689
rRNAs number	18
tRNAs number	65

Figure S1. The complete genome and features of *Streptomyces* sp. DSS69. The seven circles (inner to outer) represent the scale, GC skew, the GC content, the COG to each CDS (4th and 7th circles) and the positions of CDS, tRNA and rRNA on the genome (5th and 6th circles).

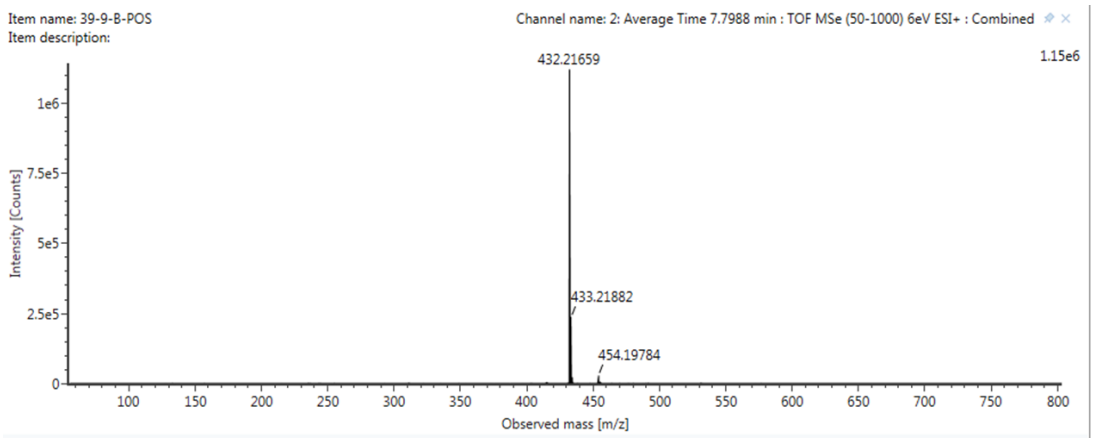


Figure S2. HR-ESI-MS spectra of compound **1**.

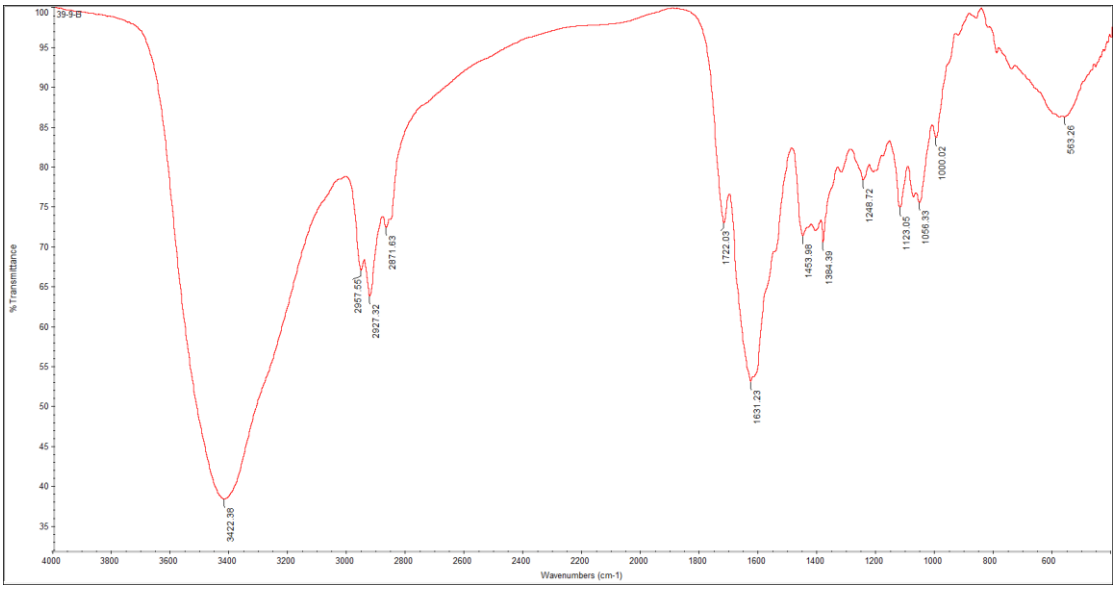


Figure S3. IR spectra of compound **1**.

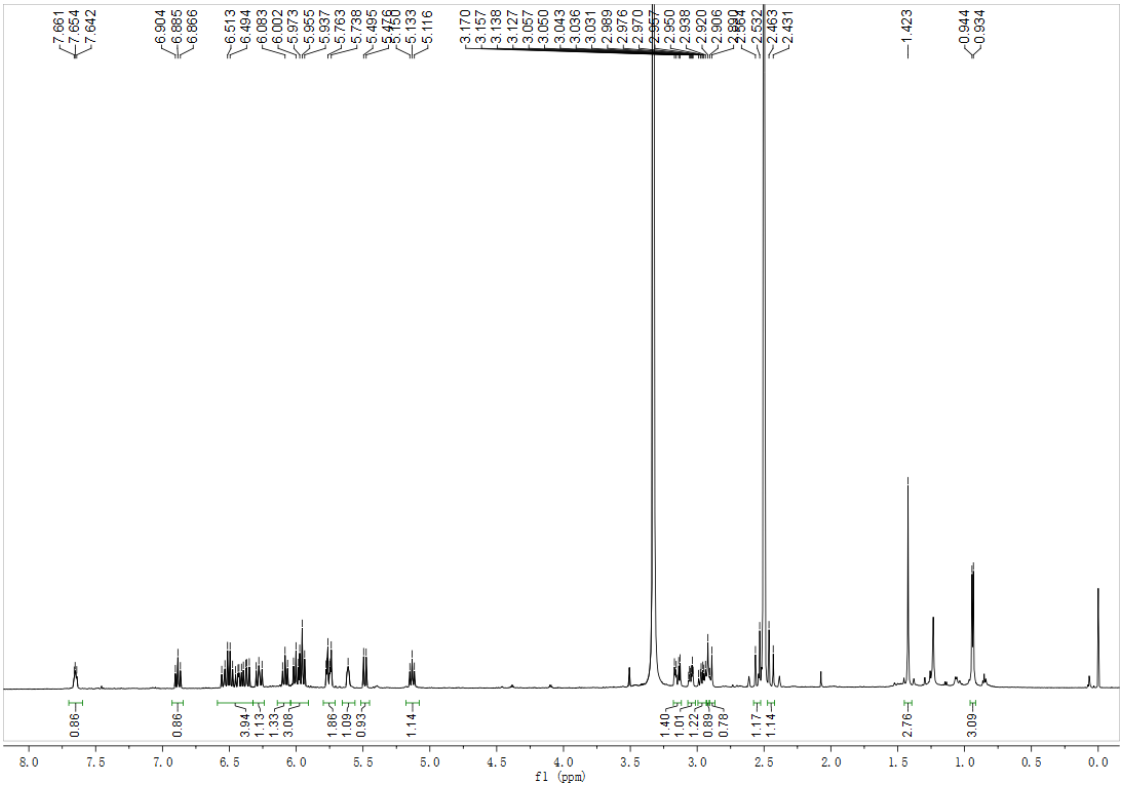


Figure S4. ^{1}H NMR spectrum of compound **1** recorded in DMSO-d_6 (600 MHz).

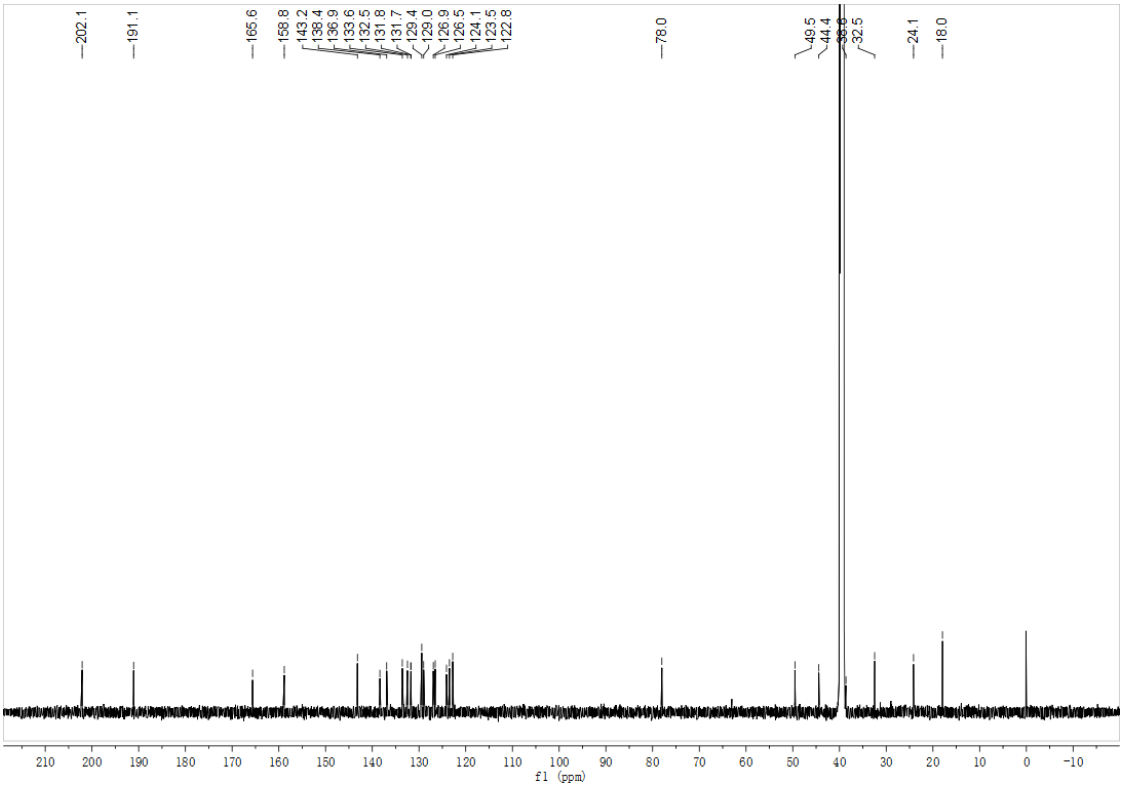


Figure S5. ^{13}C NMR spectrum of compound **1** recorded in DMSO-d_6 (150 MHz).

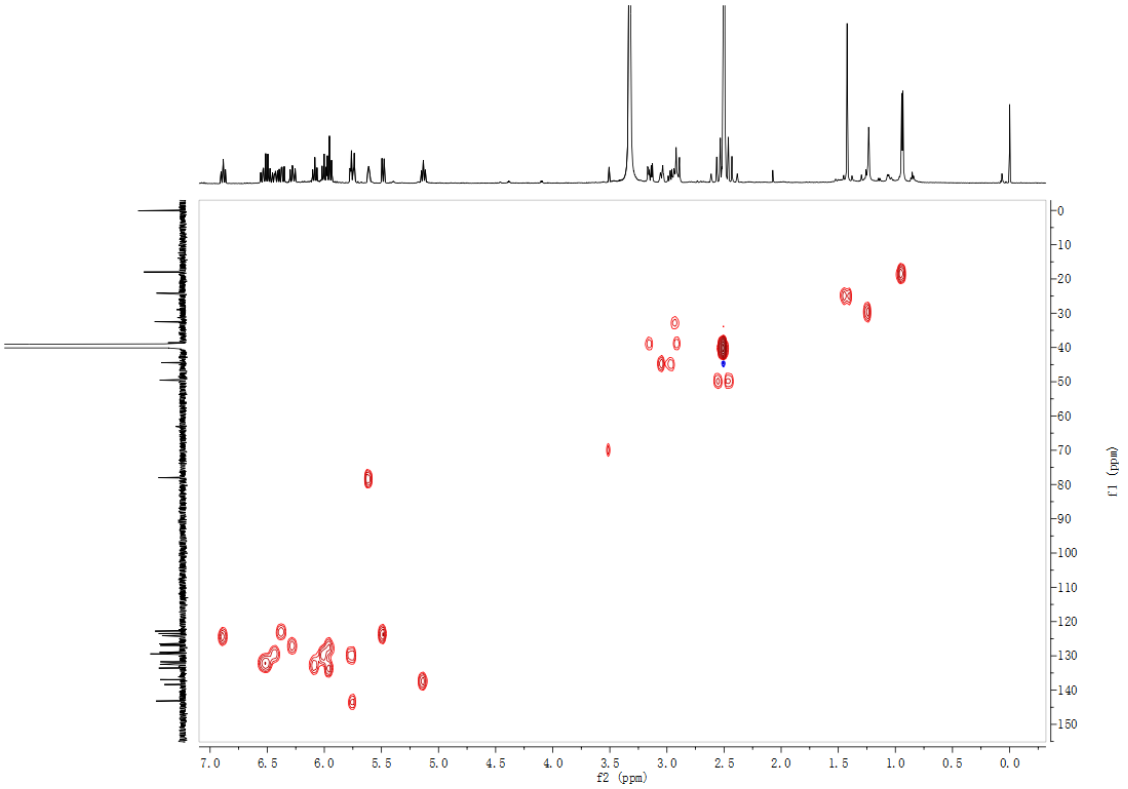


Figure S6. HSQC spectrum of compound **1** recorded in DMSO-d_6 (600 MHz).

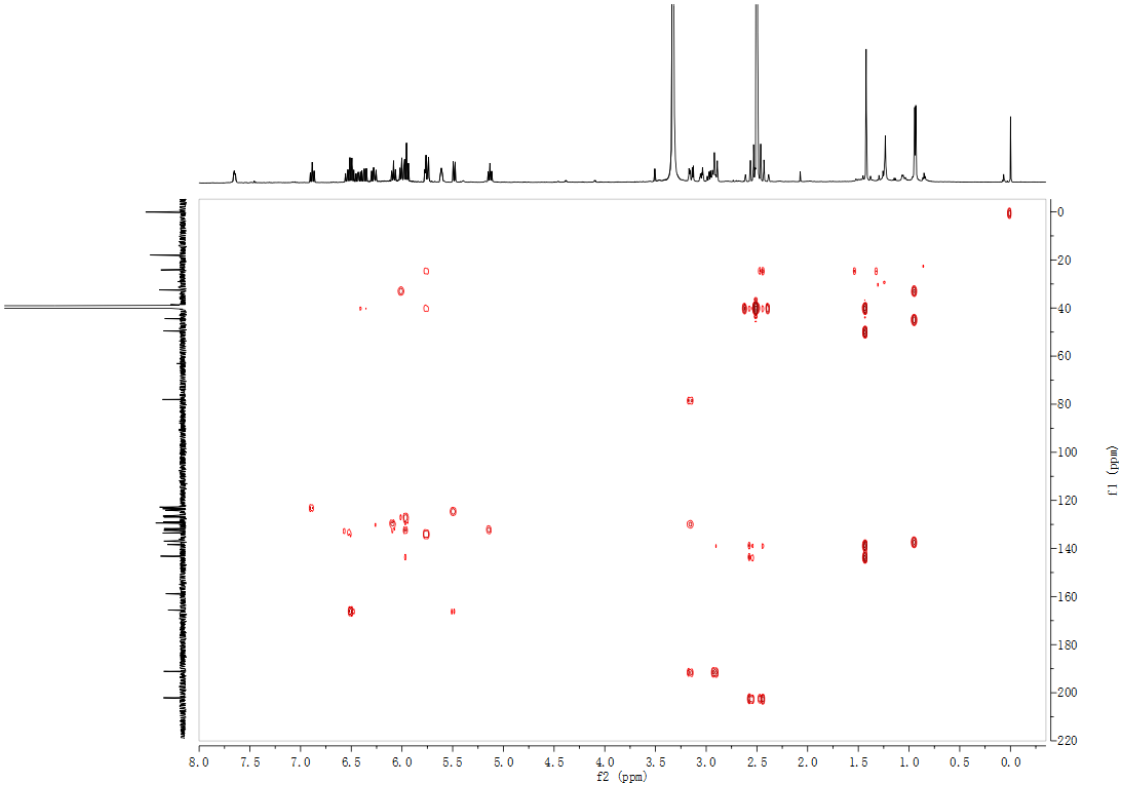


Figure S7. HMBC spectrum of compound **1** recorded in DMSO-d_6 (600 MHz).

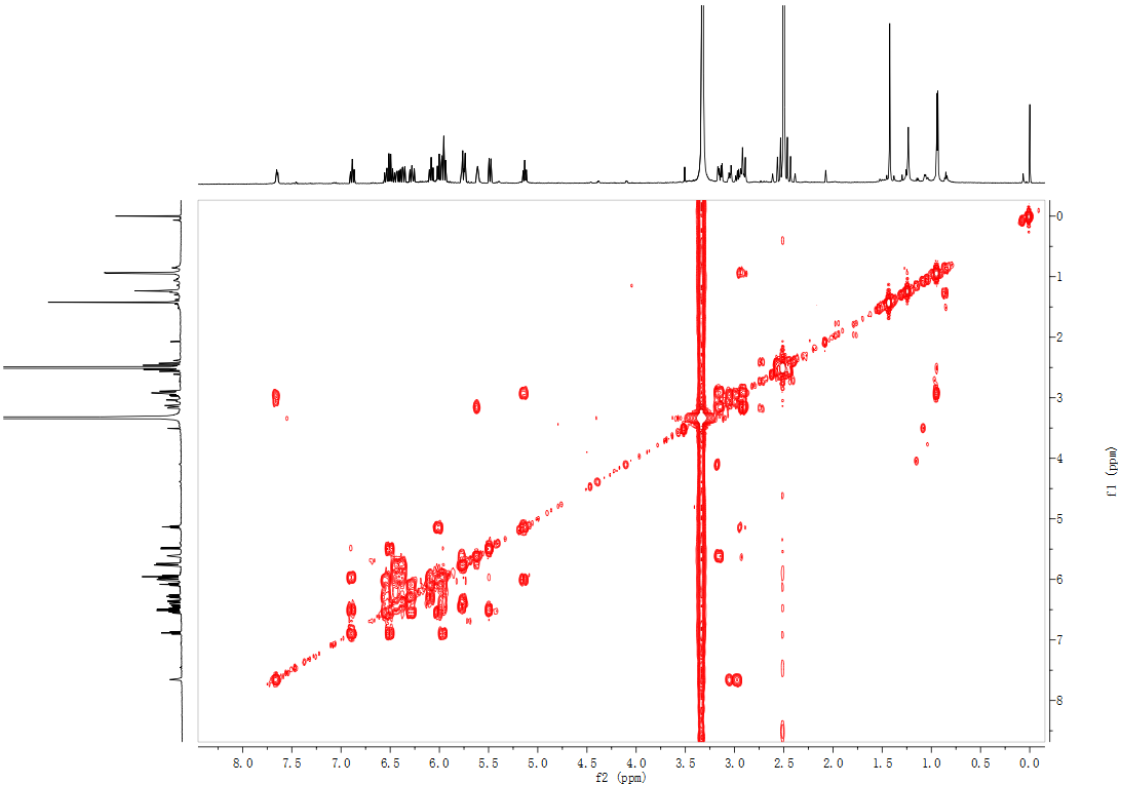


Figure S8. ^{1}H-^{1}H COSY spectrum of compound **1** recorded in DMSO-d_6 (600 MHz).

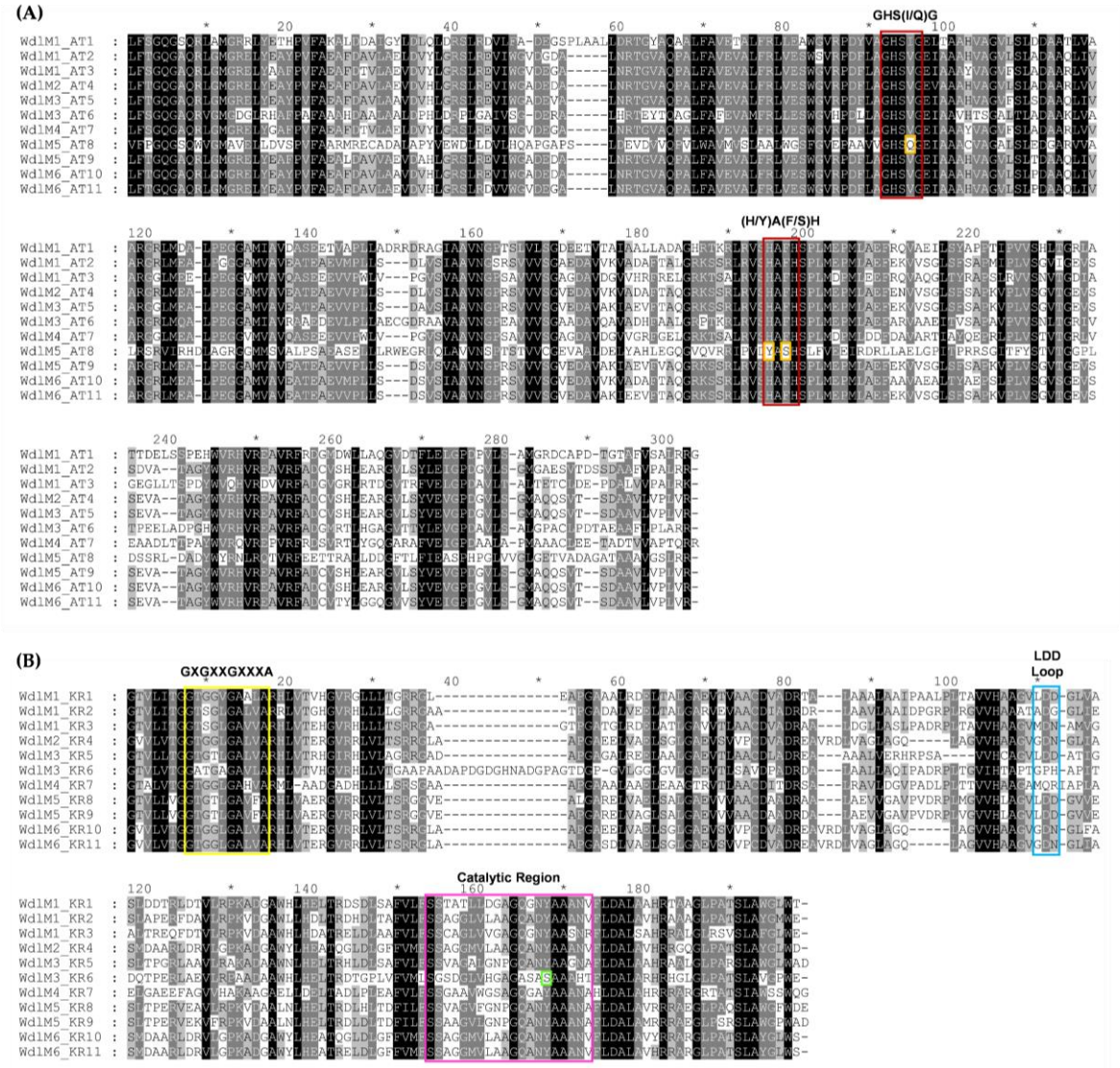


Figure S9. Multiple sequence alignment of AT domains and KR domains. **(A)** Alignment of AT domains. Red boxes indicate the characteristic residues for substrate recognition. AT domains in module 8 is predicted to be methylmalonyl-CoA-specific AT, which possess GHSQG and YASH motifs that are distinctly different with other malonyl-CoA specific ATs (indicated in yellow boxes). **(B)** Yellow box indicates conserved motif for an NADP(H) binding site. Blue box indicates LDD motif. The catalytic region is indicates as pink box. The KR domain in module 6 is predicted to be the C1 subtype, which laks the Y motif (indicated in green box).

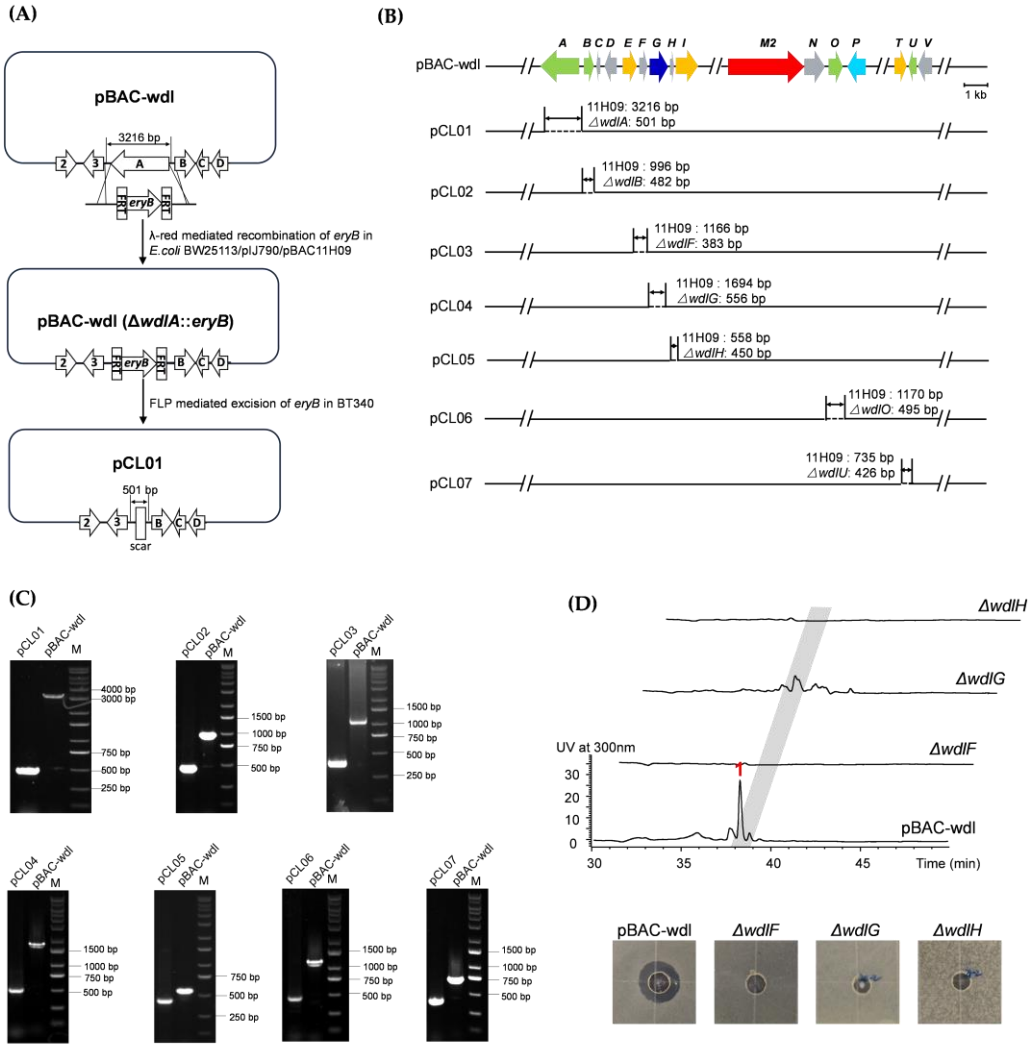


Figure S10. Disruptions of weddellamycin biosynthetic genes via PCR-targeting and PCR verification. **(A)** Schematic representation for inactivation of *wdlA* as an example. **(B)** Genetic map showing the *wdl* biosynthetic gene cluster (pBAC-wdl) and the disrupted regions for all mutants. Deleted regions are shown with dotted lines. PCR amplifications were designed for confirming each plasmid, and the amplified regions are shown as double-headed arrows with the expected sizes shown for the pBAC-wdl and mutants. **(C)** PCR vertification of the gene deletion mutants compared to original one pBAC-wdl. M is the 1 kb Plus DNA Ladder (GenStar) and numbers next to the gels are sizes of the indicated bands in bp. (D) HPLC profiles of *S. lividans GX28*/pBAC-wdl and the gene deletion mutants *ΔwdlF*, *ΔwdlG* and *ΔwdlH*. Crude extract (20 μL) was added to the central wells in the agar plates premixed with *B. altitudinis* as an indicator. Biological activity was indicated by the zones of growth inhibition after 24 h of incubation at 37℃.

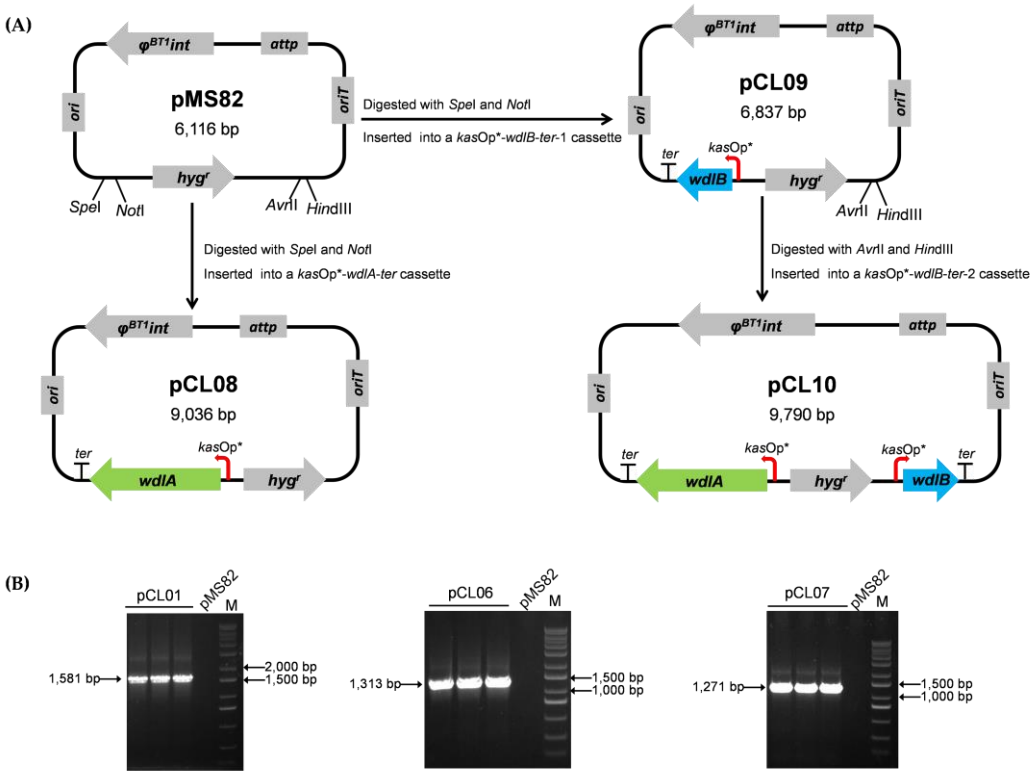


Figure S11. Schematic maps of overexpression plasmids and PCR verification. **(A)** After linearizing the pMS82 plasmid using *Not*I and *Spe*I restriction sites, the fragments *kasO*p*-*wdlA-ter* and *kasO*p*-*wdlB-ter*-1 were inserted into the plasmid using a one-step cloning method, resulting in overexpression plasmids pCL08 and pCL09. The plasmid pCL10 was obtained by linearizing pCL08 through *Avr*II and *Hin*dIII double digestion, and then inserting the fragment of *kasO*p*-*wdlB-ter*-2 using the same one-step cloning method. **(B)** PCR vertification of the overexpression plasmids compared to original one pMS82. M is the 1 kb Plus DNA Ladder (GenStar) and numbers next to the gels are sizes of the indicated bands in bp.

References

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