Engineering the acyltransferase domain of epothilone polyketide synthase to alter the substrate specificity

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Table S1. Engineered module-swap fusion sites of hybrid epothilone PKSs. (EPOM3 = blue, intermodular linker between EPOM3 and EPOM4 = blue, EPOM7 = pink, intermodular linker between EPOM7 and EPOM8 = pink, ERYM6 = red, RAPM4 = sky blue, RAPM10 = deep pink, intermodular linker between EPOM4 and EPOM5 = orange, EPOM5 = light green)

MMR2044	RGFAEQGLDSLMAVEIRKRLQGELGMPLSATLAFDHPTVERLVE
(EPOM7)	YLLSQALELQDRTDVRSARLPATEEPIAIVGIGCRFPGGAGTPEAFW
	ELLDDGRDAIRPLEERWALVGVDPGDDVPRWAGLLTEAIDGFDAAF
	FGIAPREARSLDPQHRLLLEVAWEGFEDAGIPPRSLVGSRTGVFVGV
	CATEYLHAAVAHQPREERDAYSTTGNMLSIAAGRLSYTLGLQGPCL
	TVDTACSSSLVAIHLACRSLRARESDLALAGGVNMLLSPDTMRALA
	RTQALSPNGRCQTFDASANGFVRGEGCGLIVLKRLSDARRDGDRIW
	ALIRGSAINQDGRSTGLTAPNVLAQGALLREALRNAGVEAEAIGYIE
	THGAATSLGDPIEIEALRAVVGPARADGARCVLGAVKTNLGHLEGA
	AGVAGLIKATLSLHHERIPRNLNFRTLNPRIRIEGTALELATEPVPWPR
	TGRTRFAGVSSFGMSGTNAHVVLEEAPAVEPEAAAPERAAELFVLS
	AKSVAALDAQAARLRDHLEKHVELGLGDVAFSLTTTRSAMEHRLA
	VAASSREALRGALSAAAQGHTPPGAVRGRASGGSAPKVVFVFPGQ
	GSQWVGMGRKLMAEEPVFRAALEGCDRAIEAEAGWSLLGELSAD
	EAASQLGRIDVVQPVLFAMEVALSALWRSWGVEPEAVVGHSMGEV
	AAAHVAGALSLEDAVAIICRRSRLLRRISGQGEMALVELSLEEAEAA
	LRGHEGRLSVAVSNSPRSTVLAGEPAALSEVLAALTAKGVFWRQVK
	VDVASHSPQVDPLREELVAALGAIRPRAAAVPMRSTVTGGVIAGPEL
	GASYWAGNLRQPVRFAAAARALLEGGPTLFIEMSPHPILVPPLDEIQ
	TAVEQGGAAVGSLRRGQDERATLLEALGTLWASGYPVSWARLFPAG
	GRRVPLPTYPWQHERCWIEVEPEARRLAAADPTKDWFYRTDWPEV
	PRAAPKSETAHGSWLLLADRGGVGEAVAAALSTRGLSCTVLHASA
	DASTVAEQVSEAASRRNDWQGVLYLWGLDAVVDAGASADDVSEA
	TRRATAPVLGLVRFLSAAPHPPRFWVVTRGACTVGGEPEVSLCQAA
	LWGLARVVALEHPAAWGGLVDLDPQKSPTEIEPLVAELLSPDAEDQL
	AFRSGRRHAARLVAAPPEGDVAPISLSAEGSYLVTGGLGGLGLLVAR
	WLVERGARHLVLTSRHGLPERQASGGEQPPEARARIAAVEGLEAQG
	ARVTVAAVDVAEADPMTALLAAIEPPLRGVVHAAGVFPVRPLAETD
	EALLESVLRPKVAGSWLLHRLLRDRPLDLFVLFSSGAAVWGGKGQ
	GAYAAANAFLDGLAHHRRARSLPALSLAWGLWAEGGMVDAKAHA
	RLSDIGVLPMATGPALSALERLVKTSAVQRSVTRMDWTRFAPVYAA
	RGRRNLLSALVAEDERTASPPVPTANRIWRGLSVAESRSALYELVRGI
	AARVLGFADPGALDVGRGFAEQGLDSLMALEIRNRLQRELGERLSA
	TLAFDHPTVERLVAHLLTDVLKLEDRSDTRHIRSVAADEPIAIVGAA
	CRFPGGVEDLESYWQLLAEGVVVSAEVPADRWDAADWYDPD
MMR2024	RGFAEQGLDSLMAVEIRKRLQGELGMPLSATLAFDHPTVERLVE
(ERYM6)	YLLSQA <u>LELQDRTDVRSARLPATE</u> DPIAIVGMACRFPGGVHNPGEL
	WEFIVGGGDAVTEMPTDRGWDLDALFDPDPQRHGTSYSRHGAFLD

	GAADFDAAFFGISPREALAMDPQQRQVLETTWELFENAGIDPHSLR
	GSDTGVFLGAAYQGYGQDAVVPEDSEGYLLTGNSSAVVSGRVAYVL
	GLEGPAVTVDTACSSSLVALHSACGSLRDGDCGLAVAGGVSVMAGP
	EVFTEFSRQGGLAVDGRCKAFSAEADGFGFAEGVAVVLLQRLSDAR
	RAGRQVLGVVAGSAINQDGASNGLAAPSGVAQQRVIRKAWARAGI
	TGADVAVVEAHGTGTRLGDPVEASALLATYGKSRGSSGPVLLGSVK
	SNIGHAQAAAGVAGVIKVVLGLNRGLVPPMLCRGERSPLIEWSSGG
	VELAEAVSPWPPAADGVRRAGVSAFGVSGTNAHVIIAEPPEPEPLPE
	PGPVGVLAAANSVPVLLSARTETALAAQARLLESAVDDSVPLTALA
	SALATGRAHLPRRAALLAGDHEQLRGQLRAVAEGVAAPGATTGTAS
	AGGVVFVFPGQGAQWEGMARGLLSVPVFAESIAECDAVLSEVAGFS
	ASEVLEQRPDAPSLERVDVVQPVLFSVMVSLARLWGACGVSPSAVI
	GHSQGEIAAAVVAGVLSLEDGVRVVALRAKALRALAGKGGMVSLA
	APGERARALIAPWEDRISVAAVNSPSSVVVSGDPEALAELVARCEDE
	GVRAKTLPVDYASHSRHVEEIRETILADLDGISARRAAIPLYSTLHGE
	RRDGADMGPRYWYDNLRSQVRFDEAVSAAVADGHATFVEMSPHP
	VLTAAVQEIAADAVAIGSLHRDTAEEHLIAELARAHVHGVAVDWRN
	VFPAAPPVALPNYPFEPQRYWLAPEVSDQLADSRYRVDWRPLATTP
	VDLEGGFLVHGSAPESLTSAVEKAGGRVVPVASADREALAAALREV
	PGEVAGVLSVHTGAATHLALHQSLGEAGVRAPLWLVTSRAVALGES
	EPVDPEQAMVWGLGRVMGLETPERWGGLVDLPAEPAPGDGEAFVA
	CLGADGHEDQVAIRDHARYGRRLVRAPLGTRESSWEPAGTALVTGG
	TGALGGHVARHLARCGVEDLVLVSRRGVDAPGAAELEAELVALGA
	KTTITACDVADREQLSKLLEELRGQGRPVRTVVHTAGVPESRPLHEI
	GELESVCAAKVTGARLLDELCPDAETFVLFSSGAGVWGSANLGAY
	SAANAYLDALAHRRRAEGRAATSVAWGAWAGEGMATGDLEGLTR
	RGLRPMAPERAIRALHQALDNGDTCVSIADVDWERFAVGFTAARPR
	PLLDELVTPAVGAVPAVQAAPAREMTSQELLEFTHSHVAAILGHSSPD
	AVGQDQPFTELGFDSLTAVGLRNQLQQATGLALPATLVFEHPTVRRL
	ADHIGQQLLKLEDRSDTQHVWSLASDEPIAIVGAACRFPGGVEDLE
	SYWQLLAEGVVVSAEVPADRWDAADWYDPD
MMR2027	RGFAEQGLDSLMAVEIRKRLQGELGMPLSATLAFDHPTVERLVE
(RAPM4)	YLLSQALELQDRTDVRSARLPATEEPLAIVGMACRLPGGVSSPEDL
	WRLVESGTDAVSGFPTDRGWDVENLYDSDPEAAGKSYCVQGGFLD
	TAAGFDAGFFGISPREALAMDPQQRLLLEVSWEAFERAGIEPGSVR
	GSDTGVFIGAFPVGYGAGFDREGYGATSGPSVLSGRVSYVFGLEGP
	AITMDTACSSSLVALHLAAQALRNGECSMALAGGVTVMATPEVFTE
	FARQRGLASDGRCKAFADSADGAGFSEGAGLLLVERLSDARRNGH
	QVLAVVRGSAVNQDGASNGLTAPNGPSQQRVIRAALSNAGLSTADV
	DVVEAHGTGTTLGDPIEAQALLATYGQDREQPLLLGSLKSNIGHTQ
	AASGVSGVIKMVMALRHGFVPRTLHVDEPSRHVDWAAGAVELVRE
	NQPWPGTDRPRRAGVSSFGVSGTNAHVVLESAPPAQPAEEEQPVET
	PVVASDVLPLVISAKTQPALTEHEDRLRAYLAASPGADTRAVASTLA
	VTRSVFEHRAVLLGDDAVTGTAVTDPRVVFVFPGQGWQWLGMGSA

	LRDSSVVFAERMAECAAALSEFVDWDLFAVLDDPAVVDRVDVVQP
	ASWAVMVSLAAVWQAAGVRPDAVIGHSQGEIAAACVAGAVSLRDA
	ARIVTLRSQAIARGLAGRAAMASVALPAHEIELVDGAWIAAHNGPA
	STVIAGTPEAVDHVLTAHEARGVRVRRITVDYASHTPHVELIRDELL
	GITAGIGSQPPVVPWLSTVDGSWVDSPLDGEYWYRNLREPVGFHPA
	VSQLQAQGDAVFVEVSASPVLLQAMDDDVVTVATLRRDDGDATR
	MLTALAQAYVHGVTVDWPAILGTTTARVLDLPTYAFQHQRYWVKS
	VDRAAADGHPLLGAVVELPESDGVLLTGRVSLATHAWLADHAVWG
	RVLLPGTAFVELVVHAAGEVGCDVVDELVIETPLLLPQTGGVQLSV
	SVGEADESGHRVVTVFSRADNADTWTRHVSATVRVSDTTVPPSDLT
	AWPPAQAKPVDVAGFYDQLTGMGYEYGPAFQGLQAAWRDGDTVF
	AEVALAEEOVREAARYAVHPALLDAALHACTLNASDAEVGVGLPFS
	WNGVRVHAGGSAMLRVAVTOAADGWSVRVADDIGRPVASVGSLVT
	RPVTADALGSAADDLLALTWAGIPTPQQTGLTVGRFEELVSDGDVP
	VPEVAVFTALPDNDDDPLEQTRKLTGQVLQAVQEWLGGERFSDSTL
	VVRTGTGLAAAAVSGWMRSAOSEHPGRFVLVESDDDALAPDOLAA
	AVGLDEPRLRISDGRFEAPRLTRTHAAEPESEKVWDPDGTVLITGGS
	GVLAGIAARHLVAERGVRHLLLLSRSAPDEALINQLGELGARVETA
	ACDVSDRAALAOVLAGVSPEHPLTAVIHTAGALDDGVVESLTAQRL
	DAVLRPKADGAWNLHELTRDADLAAFVMYSSAAGVLGSAGQANY
	AAANAFVDALAEORRAEGLPALAVAWGLWEDASGLTADLTDTDRD
	RIRRGGLRAISAEYGMGLFDSASRHSEPVLVGAAMEPVRDAEVPAL
	LRSLHRPIARRAASTGDSSVOWLAALAPEERAKALLRVVCDSAATV
	LGHADIDSIPVTAAFKDLGVDSLTAVDLRNSLAKATGLRLPPTLVFD
	YPTPTALAARLDELFLKLEDRSDTQHVWSLASDEPIAIVGAACRFPG
	GVEDLESYWQLLAEGVVVSAEVPADRWDAADWYDPD
MMR2026	RGFAEOGLDSLMAVEIRKRLOGELGMPLSATLAFDHPTVERLVE
(RAPM10)	YLLSOALELQDRTDVRSARLPATEEPLAIVGMACRLPGGVSSPEDL
(, ,	WRLVESGTDAISGFPTDRGWDVENLYDPDPDAPGKSYSVQGGFLD
	AAAGFDASFFGISPREALAMDPQQRLMLEVSWEAFERAGIEPGSVR
	GSDTGVFIGAYPGGYGIGADLGGFGTTAGAASVLSGRVSYFFGLEG
	PAFTVDTACSSSLVALHQAGYALRQGECSLALVGGVTVMPTPQTFV
	EFSRQRGLSADGRCKAFADAADGTGWAEGVGVLLVERLSDAQAN
	GHQILAVVRSSAVNQDGASNGLSAPNGPSQQRVIRAALSNAGLAPH
	EVDVVEAHGTGTTLGDPIEAQAVIATYGQGRGEPLLLGSLKSNVGH
	TQAAAGVSGVIKMVMALQHSMVPRTLHVDEPSRHVDWSAGAVEL
	VAENQPWPETGRPRRAGVSSFGISGTNAHVILESAPAQSVGDTAGST
	PVLVSELVPLVISAKTQPALTEHEDRLRAYLAASPGVDIRAVASTLAV
	TRSVFEHRAVLLGDETVTGTAVSDPRIVFVFPGQGWQWLGMGSAL
	RDSSVVFAERMAECAAALSEFVDWDLFAVLDDPAVVDRVDVVQPA
	SWAVMVSLAAVWQAAGVRPDAVIGHSQGEIAAACVAGAVSMRDA
	ARIVTLRSQAIARGLAGRGAMASVALPAQDVELVDGAWIAAHNGP
	ASTVIAGTPEAVDHVLTALRQRGAGAADHVDYASHTPHVELIRDEL
	LDITSDSSSQDPLVPWLSTVDGTWVDSPLDGEYWYRNLREPVGFHP

AVSQLQAQGDTVFVEVSASPVLMQAMDDDVVTVATLRRDDGDAT
RMLTALAQAYVHGVTVDWRAVLGDVPATRVLDLPTYAFQHQRYW
AEAGRSADVSAAGLDAVGHPLLGAVLAMPGSDGVMLTGRVSLATH
AWLADHAVRGSVLLPGTGFVELVVRAADEVACDVVDELIVEAPLLL
PQTGGVQLSVSVGEADESGHRAVTVFSRADSADAWVRHVSATVSV
SDTTVPTSDLTAWPPAQAKPVDVAGFYDQLTRAGYEYGPAFQGLQA
AWRDGDTVFAEVALAEEQTQDAARFAVHPAVLDAALHAGILNTPD
ADRDTVRLPFSWNHVQVHVTGSATLRVAMTRVADGWGVRVADDIG
RPVATIGSLVTRPVAADALGSAVDDLFALTWTEIPVSQQVGVTVGKF
EDLADGEVPMPDVVVFTALPDSGDPLAQTRRLTAEVLQAVQVWLA
GERFTDSTLVVRTGTGLAAAAVSGLMRSAQSEHPGRFVLVESDDDT
LTPDQLAATVGLDEPRLRVIDGRYEAPRLTRTGVAEPEPEGVWDPDG
TVLITGGSGVLAGIAARHLVAERGVRHLLLLSRSAPDEALISELAEL
GAAVVDTAVCDVSDRAGLARVLAGVSPDHPLTAVIHTAGVLDDGV
VESLTARRLDTVLRPKADGAWNLHELTRDIDLAAFVMYSSAAGVL
GSAGQGNYAVANAFVDALAEQRRAEGLPALALAWGLWEDASGLTA
KLTGTDHDRIRRSGLRTITAERGMRLFDIASRQGEPVLVATPMEPVRE
VEVPALLRLLHRPVARRAASTGDSSAQWLVGLAPEERAKALLKVV
RDSAATVLGHADARSIPATGAFKDLGVDSLTAVELRNSLTKATGLRL
PATMVFDYPTPADLAARLGDLMLKLEDRSDTQHVWSLASDEPIAIV
GAACRFPGGVEDLESYWQLLAEGVVVSAEVPADRWDAADWYDPD

Q	Epothilone yield (mg l^{-1}) ^{<i>a</i>}			
Strain	C (3)	D (4)	total	
104-1	19.85	27.67	47.52	
MMR2024 (ERYM6)	0.004	0.014	0.018	
MMR2027 (RAPM4)	nd	nd	nd	
MMR2026 (RAPM10)	nd	nd	nd	
MMR2044 (EPOM7)	0.015	0.017	0.032	
MMR2048 (EPOAT2)	8.53	26.91	35.44	
MMR2049 (EPOAT6)	6.68	30.04	36.72	
MMR2017 (EPOAT7)	6.11	28.30	34.41	
MMR2016 (EPOAT8)	7.63	29.72	37.35	
MMR2018 (RAPAT1)	< 0.001	0.017	0.017	
MMR2012 (ERYAT6)	< 0.001	< 0.001	< 0.001	
MMR2029 (EPOAT3)	55.08	nd	55.08	
MMR2020 (EPOAT9)	47.27	nd	47.27	
MMR2021 (EPOAT5)	42.53	nd	42.53	
MMR2033 (A185T)	59.77	nd	59.77	
MMR2034 (I209A)	47.11	nd	47.11	
MMR2035 (F310S)	20.34	11.26	31.60	
MMR2039 (V383L)	46.90	nd	46.90	
MMR2040 (G426R)	46.57	nd	46.57	
MMR2041 (F310S-H308V)	15.35	11.63	26.98	
MMR2042 (F310S-H308Y)	24.99	8.29	33.28	
MMR2055 (S310F)	50.34	nd	50.34	
MMR2037	nd	nd	nd	
MMR2019	22.49	28.13	50.62	
MMR2038	25.33	28.33	53.66	
MMR2053	nd	nd	nd	
MMR2054	19.61	22.14	41.75	

 Table S2. Yields of epothilones in mutants of Schlegelella brevitalea DSM 7029.

nd not detected

a The yields of epothilones are averages of three biological replicates under identical cultivation conditions.

Table S3. Alignment of nine amino acids that differ in EPOAT3 and EPOAT4. Nine amino acids in an MMCoA/MCoA-specific AT domain are shown in red; nine amino acids in MCoA-specific AT domains are shown in green; nine amino acids in MMCoA-specific AT domains are shown in orange. Four amino acids (Pro165, Gln175, Val204, and Ala205) that are identical in three AT domains (EPOAT4, EPOAT5, and EPOAT9) are highlighted in yellow.

M/MM	М	М	М	ММ	ММ	ММ	ММ	ММ	MM
EPOAT4	EPOAT3	EPOAT5	EPOAT9						
Pro165	Α	P	P	E	E	E	E	Р	1
GIn175	E	Q	Q	R	R	R	R	R	R
Thr185	Α	Α	Α	Α	Α	Α	Α	S	Α
Val204	L	L	V	V	V	V	V	V	V
Ala205	v	L	A	I.	V	V	V	I	V
Ala209	I	I	L	М	М	М	М	Q	Q
Ser310	F	F	F	S	S	S	S	S	S
Leu383	v	v	v	V	V	I.	I.	V	V
Arg426	G	Α	G	Α	Α	т	т	R	Q

Primer	Sequence (5'-3') *	Use	
JM01-F	<i>GACTACCGGGCGGCATATCA</i> TGGGTGCTGCA CACCCTTAC	Amplification of upstream homologous arm for <i>epoAT4</i>	
JM01-R	GCTCATCCCGAACGAGCTCA		
JM02-F	<i>TGAGCTCGTTCGGGATGAGC</i> CTGCCGACCTA TCCGTGGCA	Amplification of downstream homologous arm for <i>epoAT4</i>	
JM02-R	TACATCGACGAGCAGATGCT		
JM03-F	<i>TGAGCTCGTTCGGGATGAGC</i> GGCACCAACGC CCATGTCGT	Amplification of <i>epoAT2</i>	
JM03-R	CGGTACCCGCCGCCCCCG		
JM04-F	<i>TGAGCTCGTTCGGGATGAGC</i> GGGACCAACGC GCACGTGGT	Amplification of <i>epoAT6</i>	
JM04-R	CGGCACGCGACGGAGGCCCG		
JM05-F	<i>TGAGCTCGTTCGGGATGAGC</i> GGGACCAACGC GCATGTGGT	Amplification of <i>epoAT7</i>	
JM05-R	CGGAACCCGCCTGCCGCCCG		
JM06-F	<i>TGAGCTCGTTCGGGATGAGC</i> GGGACCAACG CGCATGTGGT	Amplification of <i>epoAT8</i>	
JM06-R	CGGAACCCGCCTGCCGCCCG		
JM07-F	<i>TGAGCTCGTTCGGGATGAGC</i> GGTACGAACG CCCACGTCAT	Amplification of <i>rapAT1</i>	
JM07-R	GTCCAGCACCCGGGCTGCGG		
JM08-F	<i>TGAGCTCGTTCGGGATGAGC</i> GGGACGAACG CGCACGTGAT	Amplification of <i>ervAT6</i>	
JM08-R	CGCCACCGGAGGTGCCGCCG		
JM09-F	<i>TGAGCTCGTTCGGGATGAGC</i> GGAACGAACG CGCACGTGGT	Amplification of <i>epoAT3</i>	
JM09-R	CGGCACCCGCCGCCCAGCCG		
JM10-F	<i>TGAGCTCGTTCGGGATGAGC</i> GGAACCAACG TGCATGTCGT	Amplification of <i>epoAT5</i>	
JM10-R	AGCCACGCGGCGCGCGCGCCAT		
JM11-F	<i>TGAGCTCGTTCGGGATGAGC</i> GGCACCAACG TCCATGTCGT	Amplification of <i>epoAT9</i>	
JM11-R	CGGTACCCGCCGTCCGCCCG		
JM12-F	CCGAAGAGGTCGGCAAAGTC	<i>epoAT4</i> replacement verification for upstream fragment	

Table S4. Primers used for the construction of plasmids and verification of mutant strains.

JM12-R	CGCCTTTGGTCACGTAAGTC	<i>epoAT4</i> replacement verification for downstream fragment
JM13-R	GCCTGCAGATGATCTCGTAG	<i>epoAT2</i> verification for upstream fragment
JM13-F	ACAACCTCAGGCAGCCTGTG	<i>epoAT2</i> verification for downstream fragment
JM14-R	AGCTGCGAGGTGGTCTCATC	<i>epoAT6</i> verification for upstream fragment
JM14-F	ACGAGCGCCTGTCCATGTTG	<i>epoAT6</i> verification for downstream fragment
JM15-R	CCAAGCTCGACATGCTTCTC	<i>eryAT7 and epoAT8</i> verification for upstream fragment
JM15-F	CATGGGCCGAAAGCTCATGG	<i>eryAT7 and epoAT8</i> verification for downstream fragment
JM16-R	CCAGCTCCGAATCCACTACG	<i>rapAT1</i> verification for upstream fragment
JM16-F	GGTACCGGAACTTGCGTGAG	<i>rapAT1</i> verification for downstream fragment
JM17-R	CCAATGCCGTCAACGGAACC	<i>eryAT6</i> verification for upstream fragment
JM17-F	GCCACCTTCGTCGAGATGAG	<i>eryAT6</i> verification for downstream fragment
JM18-R	CTGTCCGGTGAACAGCAACG	<i>epoAT3</i> verification for upstream fragment
JM18-F	TCGCTGGTGAGCAACCTGAG	<i>epoAT3</i> verification for downstream fragment
JM19-R	AAGCCAGCTTACCGCGTGAG	<i>epoAT5</i> verification for upstream fragment
JM19-F	AGTATTGGGTCCGGCATGTG	<i>epoAT5</i> verification for downstream fragment
JM20-R	GAACAGGAAGGCGAGCTTGC	<i>epoAT9</i> verification for upstream fragment
JM20-F	GAAATTCGTGCAGCAGATCG	<i>epoAT9</i> verification for downstream fragment
JM21-F	<i>CATTGGTAACTCGAGCTGTG</i> GTGGTCACGG ACGAGCTGAG	Amplification of upstream homologous arm for <i>epo</i> -
JM21-R	CTCTGTCGCCGGCAACCGAG	module4
JM22-F	<i>TCCGGTCGGTGGCGGCGGAT</i> GAGCCCATCG CCATCGTGGG	Amplification of downstream homologous arm for <i>epo-</i>
JM22-R	GGGATACGCACGAAGAGGCT	<i>module4</i> (for <i>epo-module7</i>)

JM23-F	CTGAAGCTGGAGGATCGCAG	Amplification of downstream homologous arm for <i>epo-</i> <i>module4</i> (for <i>ery-module7</i> , <i>rap-</i> <i>module4</i> and <i>rap-module10</i>)	
JM23-R	GGGATACGCACGAAGAGGCT		
JM24-F	<i>CTCGGTTGCCGGCGACAGAG</i> GAGCCGATCG CCATCGTGGG	Amplification of <i>epo-module7</i>	
JM24-R	ATCCGCCGCCACCGACCGGA		
JM25-F	CTCGGTTGCCGGCGACAGAGGACCCGATCG CGATCGTCGG	Amplification of any module6	
JM25-R	<i>CTGCGATCCTCCAGCTTCAG</i> GAGCTGCTGTC CTATGTGGT	Amphilication of ery-moduleo	
JM26-F	<i>CTCGGTTGCCGGCGACAGAG</i> GAGCCGTTGG CGATTGTGGG	Amplification of your we deled	
JM26-R	<i>CTGCGATCCTCCAGCTTCAG</i> GAACAACTCG TCCAGCCGGG	Ampinication of <i>rap-moaule4</i>	
JM27-F	CTCGGTTGCCGGCGACAGAGGAGCCGCTGG CGATCGTGGG		
JM27-R	CTGCGATCCTCCAGCTTCAGCATGAGGTCG CCCAGCCGGG	Amplification of <i>rap-module10</i>	
JM28-F	TCGTTGTTGCTCGACGAGAC	<i>epo-module4</i> replacement verification for upstream fragment	
JM28-R	CATGCCGGACCCAATACTCG	<i>epo-module4</i> replacement verification for downstream fragment	
JM29-R	CGCTTGAGCACGATCAGACC	<i>epo-module7</i> verification for upstream fragment	
JM29-F	TGGCTCTGGAGATCCGTAAC	<i>epo-module7</i> verification for downstream fragment	
JM30-R	ACCCACACGCCGAATGCAAG	<i>ery-module6</i> verification for upstream fragment	
JM30-F	CGCAGGAGTTGCTGGAGTTC	<i>ery-module6</i> verification for downstream fragment	
JM31-R	CTGCACGCAATACGACTTCC	<i>rap-module4</i> verification for upstream fragment	
JM31-F	CTACCGGTGACTCGTCAGTG	<i>rap-module4</i> verification for downstream fragment	
JM32-R	CCTGCACGCTGTAGGACTTC	<i>rap-module10</i> verification for upstream fragment	
JM32-F	CGGTGCGTTCAAGGATTTGG	<i>rap-module10</i> verification for downstream fragment	

JM33-F	<i>GACTACCGGGCGGCATATCA</i> TGGGTGCTGC ACACCCTTAC	Amplification of upstream homologous arm for <i>epoAT3</i>	
JM33-R	CTCCGCCCACATCACCTCGC	(epo-module4) in MMR2029	
JM34-F	<i>GCTAGGGAAGATGCCAGGAA</i> GGTGTTCTCG CTGGAAGATG	Amplification of downstream homologous arm for <i>epoAT3</i>	
JM34-R	AGCATCTGCTCGTCGATGTA	(epo-module4) in MMR2029	
JM35-R	GCGAGATCACCAAGGTAGTC	<i>aadA</i> verification for upstream fragment	
JM35-F	CTCCACCGCTGATGACATGC	<i>aadA</i> verification for downstream fragment	
JM36-F	ACGGCGTTCACCCAGCCCGCGCTCTTCACG GTGGAGTACG	Targeted mutagenesis of <i>epoAT3</i> (<i>epo-module4</i>) in MMR2029 to	
JM36-R	GCGGGCTGGGTGAACGCCGT	change Ala185 to Thr	
JM37-F	<i>TGGGGCGTAGAGCCGGAGCT</i> CCTGGTTGGG CATAGC <mark>GCC</mark> G	Targeted mutagenesis of <i>epoAT3</i> (<i>epo-module4</i>) in MMR2029 to	
JM37-R	AGCTCCGGCTCTACGCCCCA	change Ile209 to Ala	
JM38-F	<i>CGTGCGCACCAAGCGGCTGC</i> ATGTCTCGCA CGCG <mark>TCC</mark> CAC	Targeted mutagenesis of <i>epoAT3</i> (<i>epo-module4</i>) in MMR2029 to	
JM38-R	GCAGCCGCTTGGTGCGCACG	change Phe310 to Ser	
JM39-F	<i>GGACGTTC<mark>CTC</mark>GAAGTGGGGC</i> CCGAAGCCGA CGCTGCTCGG	Targeted mutagenesis of <i>epoAT3</i> (<i>epo-module4</i>) in MMR2029 to	
JM39-R	GCCCACTTCGAGGAACGTCC	change Val383 to Leu	
JM40-F	<i>GGC<mark>AGG</mark>CTGTGGGCCGGCGGC</i> GGCTCGGT CAGCTGGCCGG	Targeted mutagenesis of <i>epoAT3</i> (<i>epo-module4</i>) in MMR2029 to	
JM40-R	CCGCCGGCCCACAG <mark>CCT</mark> GCC	change Gly426 to Arg	
JM41-F	<i>GTCTCG<mark>GTC</mark>GCG<mark>TCC</mark>CACTC</i> GCCGCTGATG GAACCGATGC	Targeted mutagenesis of <i>epoAT3</i> (<i>epo-module4</i>) in MMR2029 to	
JM41-R	GAGTG <mark>GGA</mark> CGC <mark>GAC</mark> CGAGAC	Phe310 to Ser	
JM42-F	<i>GTCTCGTACGCGTCCCACTC</i> GCCGCTGATG GAACCGATGC	Targeted mutagenesis of <i>epoAT3</i> (<i>epo-module4</i>) in MMR2029 to	
JM42-R	GAGTG <mark>GGA</mark> CGC <mark>GTA</mark> CGAGAC	Phe310 to Ser	
JM43-F	<i>GTTCCACTCGCCGCTGATGG</i> AACCGATGCT GGAGGAGTTC	Targeted mutagenesis of <i>epoAT4</i> (<i>epo-module4</i>) in 104-1 to	
JM43-R	CCATCAGCGGCGAGTGGAAC	change Ser310 to Phe	
JM44-F	CCAACCTTGGGCACATGGAG	Verification for mutation of T^2 (one we detail to the formula T^2) in	
JM44-R	TCGCCTCCTCTGATTTCTGG	MMR2029 and mutation of epoAT4 in 104-1	

JM45-F	<i>GACTACCGGGCGGCATATCA</i> ATGGCCACTG CCGGACATTC	Amplification of upstream homologous arm for <i>epoDH9-</i> <i>ΨKR9-ER9</i>	
JM45-R	ACCCAGAAGGGGGGGGGGCCCC		
JM46-F	<i>GGGGCCACCCCTTCTGGGT</i> GAATCCAGCG TCGCCGTCCG	Amplification of downstream homologous arm for <i>epoDH9</i> -	
JM46-R	TCATTTTGCCTCGAACGCCG	ΨKR9-ER9	
JM47-F	<i>GACTACCGGGCGGCATATCA</i> ACCAAACCGC TGCATGTCTC	Amplification of upstream	
JM47-R	CTGAGCGTCGGGCAGCCGGT	nomologous arm for <i>epoEk9</i>	
JM48-F	ACCGGCTGCCCGACGCTCAGGAATCCAGCG TCGCCGTCCG	Amplification of downstream	
JM48-R	TCATTTTGCCTCGAACGCCG	homologous arm for <i>epoER9</i>	
JM49-F	<i>GCATTGGTAACTCGAGCTGT</i> GACATGGCGA TCGGATCTGG	Amplification of upstream	
JM49-R	TCATTTTGCCTCGAACGCCG	nomologous arm for <i>epoDH9</i>	
JM50-F	AAAGGAATAGGGTGCTGGGAGGAGACGCTC GCGTATTGCT	Amplification of downstream homologous arm for <i>epoDH9</i>	
JM50-R	GAGTTGGACGACTCGCTGAC		
JM51-F	TGGTCGGGAGTCTTCCCTTC	Verification for inactive domains	
JM51-R	AGCAGCCCGTCATCCACAAG	deletions of epo-module9	
JM52-F	GTAGCACCTGAAGTCAGCCCACACGCCTC TAGAACCCATC	Amplification of upstream homologous arm for <i>\VKR9</i> -	
JM52-R	ACCGCGCAGCGCGACCTCCT	KR9-linker	
JM53-F	AGGAGGTCGCGCTGCGCGGTGGCACCTAC CTTGTGACCGG	Amplification of downstream homologous arm for $\Psi KR9$ -	
JM53-R	GGGCTGACTTCAGGTGCTAC	KR9-linker	
JM54-F	AGGAGGTCGCGCTGCGCGGTGGGCGCCGG CGCGCAGCGCG	Amplification of <i>WKR3-KR3</i> -	
JM54-R	CCGGTCACAAGGTAGGTGCCCTCCGCAGA CAGCGACACCG	linker	
JM55-F	CAGATGTCGATGCCGATGCC	Verification for <i>\VKR9-KR9-</i>	
JM55-R	ACAAGACCTGCCGCATGCAC	linker deletion and replacement	

* Homologous arms for In-Fusion cloning are in italics, and the position of point mutations leading to amino acid changes in the target proteins are highlighted in red.

Plasmid	Relevant characteristics*	Source
pJM1	p15A_ori, sacB, Apra ^R	Lab stock
pJM2	p15A_ori, sacB, Amp^R , Hyg^R	Lab stock
pJM3	p15A_ori, sacB, Amp^R , $Apra^R$	Lab stock
pJM4	p15A_ori, sacB, Spec ^R , Apra ^R	Lab stock
pJM5	pJM1 derivative containing <i>epoAT4</i> upstream and downstream	This study
	fragments, Apra ^R	
pJM6	pJM5 with $epoAT2$, $Apra^R$	This study
pJM7	pJM5 with $epoAT6$, $Apra^R$	This study
pJM8	pJM5 with $epoAT7$, $Apra^R$	This study
pJM9	pJM5 with $epoAT8$, $Apra^R$	This study
pJM10	pJM5 with <i>rapAT1</i> , <i>Apra^R</i>	This study
pJM11	pJM5 with $eryAT6$, $Apra^R$	This study
pJM12	pJM5 with $epoAT3$, $Apra^R$	This study
pJM13	pJM5 with $epoAT5$, $Apra^R$	This study
pJM14	pJM5 with $epoAT9$, $Apra^R$	This study
pJM15	pJM4 derivative containing epoAT3 (epo-module4) upstream	This study
	and downstream fragments, Spec ^R , Apra ^R	
pJM16	pJM5 with <i>epoAT3</i> mutant encoding EPOAT3 (A185T), $Apra^{R}$	This study
pJM17	pJM5 with <i>epoAT3</i> mutant encoding EPOAT3 (I209A), $Apra^{R}$	This study
pJM18	pJM5 with <i>epoAT3</i> mutant encoding EPOAT3 (F310S), $Apra^{R}$	This study
pJM19	pJM5 with <i>epoAT3</i> mutant encoding EPOAT3 (V383L), $Apra^{R}$	This study
pJM20	pJM5 with <i>epoAT3</i> mutant encoding EPOAT3 (G426R), $Apra^{R}$	This study
pJM21	pJM5 with epoAT3 mutant encoding EPOAT3 (F310S,	This study
	H308V), $Apra^R$	
pJM22	pJM5 with epoAT3 mutant encoding EPOAT3 (F310S,	This study
	H308Y), $Apra^R$	
pJM23	pJM5 with $epoAT4$ mutant encoding EPOAT4 (S310F), $Apra^{R}$	This study
pJM24	pJM1 derivative containing <i>epoDH9-\U0144KR9-ER9</i> upstream and	This study
	downstream fragments, <i>Apra^R</i>	
pJM25	pJM3 derivative containing <i>epoER9</i> upstream and downstream	This study
	fragments, Amp ^R , Apra ^R	
pJM26	pJM2 derivative containing epoDH9 upstream and	This study
	downstream fragments, <i>Amp^R</i> , <i>Hyg^R</i>	
pJM27	pJM1 derivative containing <i>WKR9-KR9-linker</i> upstream and	This study
	downstream fragments, <i>Apra^R</i>	
pJM28	pJM27 with $\Psi KR3$ -KR3-linker, Apra ^R	This study
pJM29	pJM3 derivative containing epo-module7 together with epo-	This study
	<i>module4</i> upstream and downstream fragments, Amp^R , $Apra^R$	
pJM30	pJM3 derivative containing ery-module6 together with epo-	This study
	<i>module4</i> upstream and downstream fragments, Amp^R , $Apra^R$	

 Table S5. Plasmids used in this study.

pJM31	pJM3 derivative containing <i>rap-module4</i> together with <i>epo-</i> module4 upstream and downstream fragments. Amr^R Amr^R	This study
	module4 upsileani and downstreani fragments, Amp, Apra	
pJM32	pJM3 derivative containing rap-module10 together with epo-	This study
	<i>module4</i> upstream and downstream fragments, <i>Amp^R</i> , <i>Apra^R</i>	

* Amp^R ampicillin resistance, $Apra^R$ apramycin resistance, Hyg^R hygromycin resistance, $Spec^R$ spectinomycin resistance



Figure S1. Structural models of reducing domains in wild-type and mutant EPOM9s. **a** Structural models of DH9- Ψ KR9-ER9-KR9 domains of the wild-type EPOM9. **b** Structural models of the Ψ KR9-KR9 didomain of the mutant EPOM9 (deleted DH9 and ER9 domains). **c** Ψ KR9-KR9 structural model (shown in yellow) superimposed on the DH9- Ψ KR9-ER9-KR9 model (shown in blue); the calculated RMSD = 1.980, which shows these two models are similar.



Figure S2. Engineering the epothilone PKS by whole-module swapping. The EPOM4 was replaced with EPOM7 while the natural intermodular linker between EPOM3 and EPOM4 (shown in blue) and the linker between EPOM7 and EPOM8 (shown in pink) were retained. EPOM4 was replaced with ERYM6, RAPM4, or RAPM10 while the natural intermodular linker between EPOM3 and EPOM4 (shown in blue) and the linker between EPOM3 and EPOM4 (shown in blue) and the linker between EPOM3 and EPOM4 (shown in blue) and the linker between EPOM3 and EPOM4 (shown in blue) and the linker between EPOM3 and EPOM4 (shown in blue) and the linker between EPOM3 and EPOM4 (shown in blue) and the linker between EPOM3 and EPOM4 (shown in blue) and the linker between EPOM4 and EPOM5 (shown in orange) were retained.



Figure S3. Extracted ion chromatogram (EIC) of LC–MS analyses of epothilone production in EPOM4-swap mutants. Peaks corresponding to epothilone C (selected for m/z = 478.4 and 290.2), epothilone D (selected for m/z = 492.4 and 304.2) were shown in blue and red, respectively.



Figure S4. Multiple sequence alignment of AT domains used in this study. Red arrows indicate boundaries for AT swap. EPO, epothilone PKS; ERY, erythromycin PKS; RAP, rapamycin PKS; KAL, KS-AT linker; PAL1, non-conserved N-terminal region in the post-AT linker; PAL2, conserved C-terminal region in the post-AT linker.



Figure S5. Extracted ion chromatogram (EIC) of LC–MS analyses of epothilone production in some AT-swap mutants to further confirm the production of epothilones. Peaks corresponding to epothilone C (selected for m/z = 478.4 and 290.2), epothilone D (selected for m/z = 492.4 and 304.2) were shown in blue and red, respectively.



Figure S6. Extracted ion chromatogram (EIC) of LC–MS analyses of epothilone production in AT-swap mutants MMR2012 and MMR2018. Peaks corresponding to epothilone C (selected for m/z = 478.4 and 290.2), epothilone D (selected for m/z = 492.4 and 304.2) were shown in blue and red, respectively.



Figure S7. HPLC analysis of the extracts from the parental and site-directed mutant strains.

		ΨKR-ER boundary	ER deletion point
	1 10 20	30 40	Ļ
EPOM3 EPOM7 EPOM9 EPOM2 EPOM6 EPOM5	VDLDPEESPT.EVEALVAELLSPDAE VDLDPQKSPT.EIEPLVAELLSPDAE VDLDPAQPEG.EADALLAELLADDAE VDLDPARPDG.EIGALLAELLADDAE IDLDPARRDG.EVDALLAELLADDAE VDLEPEADAARSADVILREIGRADDE	D QLAFROGRRAARLVAAP D QLAFRSGRRHAARLVAAP E EVALRGGERFVARLVH R LP E EVALRGGERFVARLVH R LP E EVALRGGERCVAR IVR R QP E EVAFRGGERRVARLVR R P T QVVFRSGKRRVGRLVKAT	EGN EGD. DAQRREKVEPAGDR ETR ETDCREKIEPAEGR PEGLLVPDAE
		ΨKR-KR-linke	er
EPOM3 EPOM7 EPOM9 EPOM2 EPOM6 EPOM5	PFRLEIDEPGALDQLVLRATGRRAPG PFRLEIDGSGVLDDLVLRATERRPPG SYRLEAGQKGTLDQLRLAPAQRRAPG	PGEVEIAVEAAGLDSIDIQI PGEVEIAVEAAGLNFLDVMR PGEVEIKVTASGLNFRTVLA	ALGVAPNDLPGEEI RAMGIYPGPGD VLGMYPG
EPOM3 EPOM7 EPOM9 EPOM2 EPOM6 EPOM5	EPSVLGRECAGRIVAVGEGVNGLVVG GPVALGAECSGRIVAMGEGVESLRIG DAGPMGGDCAGIVTAVGQGVHHLSVG	QPVIALAAGVFATHVTTSAT QDVVAVAPFSFGTHVTVDAR DAVMTLG.TLHRFVTVDAR	LVLPRPLGLSATEA MVAPRPAALTAAQA LVVRQPAGLTPAQA
EPOM3 EPOM7 EPOM9 EPOM2 EPOM6 EPOM5	AAMPLAYLTAWYALDKVAHLQAGERV AALPVAFMTAWYGLVHLGRLRAGERV ATVPVAFLTAWLALHDLGNLQRGERV	LIHAEAGGVGLCAVRWAQRV LIHSATGGTGLAAVQIARHL LIHAAAGGVGMAAVQIARWI	GAEVYATADTPEKR GAEIFATAGTPEKR GAEVFATASPSKWA
EPOM3 EPOM7 EPOM9 EPOM2 EPOM6 EPOM5	AYLAS.LGVRYVSDSRSGRFAADVHA AWLRE.QGIAHVMDSRSLDFAEQVLA ALQAMGVPRTHIASSRTLEFAETFRQ	WTDGEGVDVVLDSLSGEHID ATKGEGVDVVLNSLSGAAID VTGGRGVDVVLNALAGEFVD	SSLMVLRACGRLVK ASLSTLVPDGRFIE ASLSLLSTSGRFLE
EPOM3 EPOM7 EPOM9 EPOM2 EPOM6 EPOM5	LGRRDDCPDTQPGLPPLLRNFSFSQVI LGKTDIYADRSLGLAHFRKSLSYSAVI MGKTDIRDRAAVAAAHPGVRYRVFDI	DLRGMMLDQPARIRALLDEL DLAGLAVRRPERVAALLAEV LELAPDRTREILERVVEGFA	FGLVAADPISPLGW VVDLLARG
EPOM3 EPOM7 EPOM9 EPOM2 EPOM6 EPOM5	GLRVGGSLTPPPVETFPISRAAEAFR ALQPLPVEIFPLSRAADAFR HLRALPVHAFAITKAEAAFR	EK GENEUG RMAQRQHLGKLVLTLDDPEV PRG KMAQAQHLGKLVLALEDPDV FMAQARHQGK	A POINT 50 A APVSISA VRIRAPAESSVAVRA RIESCVPTDVTIRA VRIRVSGESGVAIRA VVLLPAPSAAPLAP
	ER-KR boundary		ΨKR-KR-linker
EPOM3 EPOM7 EPOM9 EPOM2 EPOM6 EPOM5	EGSYLVTGGLG DGTYLVTGGLG DGTYLVTGGLG DSTYLVTGGLG DGTYLVTGGLG TGTVLLTGGLG		

Figure S8. Junctions for ER domain deletion constructs. Black arrows indicate ER domain deletion points. Blue arrows indicate Ψ KR-ER and ER-KR boundaries. The Ψ KR9-KR9-linker is underlined in red. EPO: epothilone PKS.



Figure S9. Junctions for DH domain deletion constructs. Arrows indicate deletion points. BOR: borrelidin PKS [1]; RAP: rapamycin PKS.



Figure S10. PCR analysis for the confirmation of AT-swap mutants. a PCR analysis for the replacement of EPOAT4 with MMCoA-specific ATs, which resulted in MMR2016, MMR2017, MMR2012, MMR2018, MMR2048, and MMR2049. b PCR analysis for the replacement of EPOAT4 with MCoA-specific ATs, which resulted in MMR2020, MMR2021, and MMR2029. -, PCR analysis with genomic DNA from Schlegelella brevitalea 104-1, which was used as a negative control. Red arrows indicate the expected up-AT4swap and AT4swap-down fragments in seven mutant strains that were amplified by PCR using the primers JM12-F/R and JM20-F/R (Additional file 1: Table S3). The up-AT4swap and AT4swap-down fragments are 2932 bp and 3053 bp long for MMR2016 and MMR2017, 2958 bp and 2284 bp for MMR2012, 2856 bp and 2351 bp for MMR2018, 2917 bp and 2364 bp for MMR2048, 3274 bp and 2199 bp for MMR2049, 3116 bp and 2532 bp for MMR2020, 3108 bp and 2370 bp for MMR2021, and 3125 bp and 2425 bp for MMR2029. The expected DNA fragments were obtained from both 104-1 and MMR2029 (EPOAT3) because EPOAT3 and EPOAT4 differ in only nine amino acids. All PCR products were then sequenced, and the results further confirmed that EPOAT4 in the epothilone biosynthetic gene cluster was successfully replaced with non-native AT domains by double crossover.



Figure S11. PCR analysis for the confirmation of module-exchange mutants. PCR analysis for the substitution of EPOM4 with modules that harbour MMCoA-specific AT domains, which resulted in MMR2024, MMR2026, MMR2027, and MMR2044. –, PCR analysis with genomic DNA from *Schlegelella brevitalea* 104-1, which was used as a negative control. Red arrows indicate the expected up-M4swap and M4swap-down fragments in four mutant strains by PCR that were generated using the primers JM28-F/R and JM32-F/R (Additional file 1: Table S3). The up-M4swap and M4swap-down fragments are 3274 bp and 2616 bp long for MMR2024, 2908 bp and 2537 bp for MMR2026, 2907 bp and 2670 bp for MMR2027, and 3446 bp and 2502 bp for MMR2044. All PCR products were then sequenced, and the results further confirmed that EPOM4 in the epothilone biosynthetic gene cluster was successfully replaced with non-native modules by double crossover.



Figure S12. DNA sequences of the PCR products of site-directed mutants. The left column in **a-g** shows DNA sequences of the PCR products of parent strain MMR2029. The left column in **h** shows DNA sequences of the PCR products of parent strain 104-1. The right column shows DNA sequences of the PCR products of site-directed mutants. **a** MMR2029 and MMR2033 (A185T). **b** MMR2029 and MMR2034 (I209A). **c** MMR2029 and MMR2035 (F310S). **d** MMR2029 and MMR2039 (V383L). **e** MMR2029 and MMR2040 (G426R). **f** MMR2029 and MMR2041 (F310S-H308V). **g** MMR2029 and MMR2042 (F310S-H308Y). **h** 104-1 and MMR2055 (S310F). The mutated bases are highlighted with red boxes, and the corresponding bases in MMR2029 and 104-1 are highlighted with black boxes.

MMR2053

Leu Leu Ala diu Leu Leu Ala diu Leu Leu Ala dap Aap Ala 220 diu diu Val Ala 225 Aig diy diy Thr Tyr Leu Val Thr diy diy Leu diy Leu diy Leu Ala diy Thr L M9-WKR M9-WKR M9-WKR M9-WKR M9-KR → M9-KR	yaaacgaccggctcgatgaac	gtctacta	cggctcc	tcctc	cagcg	cgac	gcgcc	accgt	ggat	gaac	acto	ggcc	gcca	jacc	cacco	cga	gccag	acg	cgca	ccgg	gccta	accgad
M9-WKR M9-WKR M9-WKR M9-WKR M9-WKR M9-WKR M9-WKR M9-WKR M9-KR M9-K	210 215	Ala Asp Asp	Ala Glu	Glu Glu	Val Ala	225	Ara Gh	1 Gly	Thr Tyr	Leu	5 Val Th	hr Gly	Gly	Leu (10 Gly Gly	Leu	Gly I	eu A	IS Va	I Ala	Gly	Tro Leu
Etttgctggccgagctacttgcagatgatgccggagggggggg		M9-W	(R	old old	Full Full				111 191	LCU	101 11		Gill		M9-K	R →			ing vu	1 100	Giy	np cou
MMR2054																						
TITIGCTGGCCGAGCTACTTGCAGATGATGCCGAGGAGGAGGAGGGCGCCCTGCGCGGGGGGGG	tttgctggccgagctactt	cagatgat	gccgagg	aggag	gtcgc	acta	acaa	tggca	ccta	ctto	tgad	ccaa	caato	taa	ataa	acto	agto	tgc	gcgt	aaco	ggat	tggctd
MMMR2054 raggagggt cg cg ct gc gg t 666Cccccccccccccccccccccccccccccccccc	TTTGCTGGCCGAGCTACTTC	CAGATGAT	GCCGAGG	AGGAG	GTCGC	GCTG	GCGG	TGGCA	CCTA	CTTO	TGAC	CCGG	CGGTO	TGG	GTGG	асто	GGTC	TGC	GCGT	GGCC	GGA	госто
MMM200 270 280 200 300 310 320 330 340 350 350 350 MMR2054 Integraggit cg					٨			٨												٨		
250 270 280 290 300 310 320 330 340 350 3 MMR2054 mggagggtgtcgcgctgcgcggtc666cccc66cc6cccccccc	AAAAAAAA AAA AAAAAAA	A. AMAN	AAAA A	1. 1.	1. 1. 1	11/1	٨٨٨	MA	٨٨٨٨	1000	AA	٨٨٨	٨٨٨٨	٨٨٨	A.AA	N.A	IA A	٨٨٨		1		MAA.N
MMR2054 Iaggaggtcgcgcgcgcgcccccccccccccccccccccc		270	VVVW W	VVVV	WW	//////	WWW	UVVV	VVVVV	VVVV	WVV	VVV	Ινννν	VVVI	1 VV VV	VVV	WW	<u>UVV</u>	VVV	VW	VVVV	<u>YVW</u>
MMR2054 Iaggaggtcgcgctgcgcggt6666C6C66C6C6C6C6C6C6C6C6C6C6C6C6C6C6																						
MMR2054 aggaggtcgcgcgcgcgcgcgcccccccccccccccccc																						
YIMIKZUD34 laggaggtcgcgctgcgcgcgcgccacccaccaccaccaccaccaccaccacca																						
aggaggtcgcgcgcgcgcgcGGCGCGGCGCGCGCGCGCGCGCGCGGGGCCCGTGGGCGCCCACCGGAGGGAAACGCAGCGCCGGTGTCGCGGCCCCGGAGGGGAACGCAGCGCCGGGGGGCACCAGCGGCG																						
tcctccagcggagggggcgcacccccccccccccccccc	MMR2054																					
and du val al 225 ig div div al al 225 ig div div Aig Aig Aig Aig Aig Aig Aig Aig Aig a div val Aia Aig teu Val Aia Aig Pro Pro div Giv Aig	MMR2054	GEGCECCO	GCGCGCA	ecece	GCTCG	TGGCO	ceccc	CACCO	GAGG	GAAAC	GCAG	GCGC	CGGT	STCG	стет	сте	CGGAG	ggc	acct	acct	ttgt	gaccg
aggaggtcgcgctgcggt6666c6cc66c6c6c6c6c6c6c6c6	MMR2054	GGGCGCCC	GCGCGCG	GCGCGC	GCTCG	TGGC	GCGGG	CACCO GTGGC	GAGG	GAAAC	GCAG	GCGC	GCCAG	GTCG	GACA	GAC	GGGAG	ggc ccg	<mark>acct</mark> + ++ tgga	acct tgga	ttgt	gaccgg
aggaggtcgcgctgcggt666c6cc66c6c6c6c6c6c6cccccccc	MMR2054 aggaggtcgcgctgcgcgg tcctccagcgcgacgcgccc	GGGCGCCCC CCCGCGGGC	GCGCGCGCA CCGCGCGT		GCTCG CGAGC	TGGCC ACCGC	cocc cocc	GTGGC	GAGG		GCAG	coco	CGGT GCCAC	CAGC	GACA	GACO	CGGAG GCCTC 25	ggc ccg	acct tgga	acct tgga	ttgt aaca	gaccgg
laggaggtcgcgctgcgcggt666c6cc66c6c6c6c6c6c6ccc66cc6cccccccc	MMR2054 aggaggtcgcgctgcgcgg tcctccagcgcgaccgcgc du du va ala teo arg dy M94000000000000000000000000000000000000	GGGCGCCC CCCGCGGGG Gly Arg Arg	GGCGCGCA CGCGCGCGT CGCGCGCGT S rg Arg Ala	AGCGCGC CCGCGCC Ala Arg	GCTCG CGAGC	TGGCO ACCGO 10 Val Ala	GCCC GCGGG Ala F	GTGGC GTGGC Pro Pro	GAGG CTCC Glu G	GAAAC CTTTC 5 Iy Asn	GCAC CGTC Ala	GCGC CGCG Ala F	GCCAC GCCAC 20 Pro Val	GTCG CAGC Ser	GACA Leu S	GACO er A	GGGAG GCCTC 25 Ja Glu	ggc ccg 1 Gly	acct + ++ tgga Thr 1	acct tgga fyr Le	ttgt aacau 5 eu Val	gaccgg ctggcc Thr G
aggaggtcgcgctgcgcggtgcGGGCGCGGCGCGCGCGGCGCGG	MMR2054 aggaggtcgcgctgcgcgg tcctccagcgcgacgcgcgcc Giu Giu Val Ala Leu Arg Gly M9-WKR	GGGCGCCC CCCGCGGG 1 Gly Arg Ai	GGCGCGCA CGCGCGCGT CGCGCGCGT rg Arg Ala	AGCGCGC CGCGCC Ala Arg	GCTCG CGAGC	TGGCC ACCGC 10 Val Ala	Ala F	CACCO GTGGC Pro Pro M3-linka	Glu G	SAAAC CTTTC 5 ly Asn	GCAC CGTC Ala	GCGC CGCG Ala F	GCCAC GCCAC 20 Pro Val	GTCG CAGC Ser	CTGT GACA	GACO er A	CGGAG 3CCTC 25 Ja Glu	ggc ccg l Gly	acct + ++ tgga Thr 1	acct tgga lyr Le MS	ttgt aacar s eu Val	gaccgg ctggcc Thr G
AGGAGGTCGCGCTGCGCGCGGGGGCGCGGCGCGCGCGCGGCGCGGCG	MMR2054 aggaggtcgectgcgggg tcctccagcgcgacggccc du du val Ala Lee Arg dy M9-WKR	GGGCGCCC CCCGCGGG 1 Gly Arg Ai	GGCGCGCA CGCGCGCGT GGCGCGCGT S gg Arg Ala	AGCGCG CGCGCC Ala Arg	GCTCG CGAGC	TGGCC ACCGC 10 Val Ala	Ala F	CACCO GTGGC Pro Pro M3-linka	GGAGG CCTCC Glu G	SAAAC CTTTC 5 J	GCGTC Ala	GCGC GCG Ala F	CGGT GCCA 20 Pro Val	GTCG CAGC Ser	CTGT GACA	GACC	CGGAG GCCTC 25 Ja Glu	ggc ccg 1 Gly	acct + + + tgga Thr 1	acct tgga Tyr Le	aaca 5 eu Val O-KR	gaccgg ctggcc Thr G
	MMR2054 aggaggtcgcgcgcgcg tccctccagcgcgacgcgc du du val Ala Leu Arg dy M9-WKR	GGGCGCGCG CCCGCGCGGG Gly Arg A	GCGCGCGCA	AGCGCGC Ala Arc	GCTCG	TGGCC ACCGC 10 Val Ala	Ala F	CACCO GTGGC Pro Pro M3-linka	GAGG	SAAAC	Ala	GCGC Ala F	CGGT(GCCA(20 Pro Val	Ser Ser	CTGT	GACO er A	25 Ja Glu	ggc ccg 1 Gly	acct	acct tgga Tyr Le MS	ttgt aaca 5 20 Val O-KR	gaccgg ctggcc Thr G →
	MMR2054	GGGCGCCCC	GCGCGCGCA SCGCGCGCG Srg Arg Ala		GCTCG	TGGCC ACCGC 10 Val Ala	Ala F	CACCO GT GG C Pro Pro M3-linka	GAGGG	GAAAC	Ala	GCGC Ala F	CGGT(GCCA(20 Pro Val	STCG	CTGT GACA	er A	CGGAG	ggc ccg 1 Gly	acct Thr 1 acct	acct tgga MS acct	ttgt aacau 5 2 Val 0-KR	gaccgg Thr G → gaccgg
	MMR2054 aggaggtcgcgctgcgcgt tcctccagcgcgacgcgcg Gu Gu Vel Ala Leu Arg Gy M9-WKR	GGGCGCCCG	rg Arg Ala	ACCCCCG Ala Arg	GCTCG GCTCG GCTCG	TGGCC	Ala F	CACCO GTGGC Pro Pro M3-linka CACCO	GAGG Glu G Glu G Glu G Glu G GGAGG	GAAAC	Ala	Ala F	CGGT CGGT	Ser Ser	CTGT CTGT CTGT	CTGC GACC er A	CGGAG CGGAG CGGAG	iggc iccg i Gly iggc iGGC	acct Thr 1 acct ACCT	acct tgga Mg Acct	ttgt aaca s val o-KR	gaccgg ttgcc Thr G → gaccgg SACCGO

Figure S13. DNA sequences of the PCR products of ΨKR9-KR9-linker deletion mutant (MMR2053) and replacement mutant (MMR2054).



Figure S14. Standard curves calculated based on the peak areas of epothilone standards of different concentrations. Different concentrations of each epothilone component (1 ng/ml, 5 ng/ml, 10 ng/ml, 50 ng/ml, 100 ng/ml, 200 ng/ml, and 500 ng/ml) were used to test the standard curves.

References

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