Supplementary Information for

The Ketosynthase Domain Constrains the Design of Polyketide Synthases

*Maja Klaus[a+], Lynn Buyachuihan[a+] and Martin Grininger[a]**

[a] Institute of Organic Chemistry and Chemical Biology, Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt
[+] These authors contributed equally.
** Corresponding author: grininger@chemie.uni-frankfurt.de*

Content:

Supplementary Figures:

Supplementary Tables:

Supplementary Figures

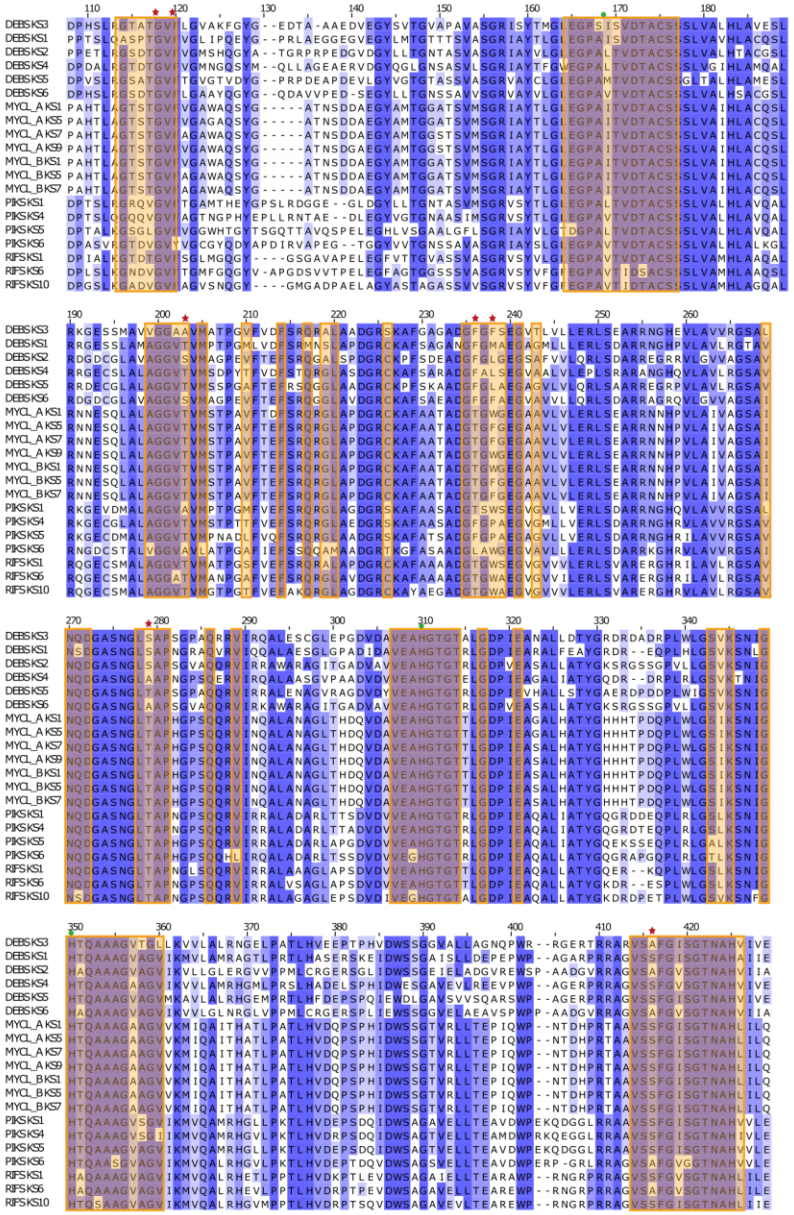

Figure S1. Sequence alignment of KS domains to identify residues for site-directed mutagenesis of DEBS KS3. Sequences were obtained from DEBS, mycolactone synthase (MYCL), PIKS, and RIFS. Residues within 12 Å of the active cysteine of DEBS KS3 (C175; Uniprot EryA2 Q03132 position 202) are highlighted in orange. Green circles- catalytic triad, red stars- residues selected for multipoint mutagenesis (A124, F126, A203, F236, F238, S279, and A416). Nomenclature of selected residues according to PDB 2OQ3: A154, F156, A230, F263, F265, S306, A441.

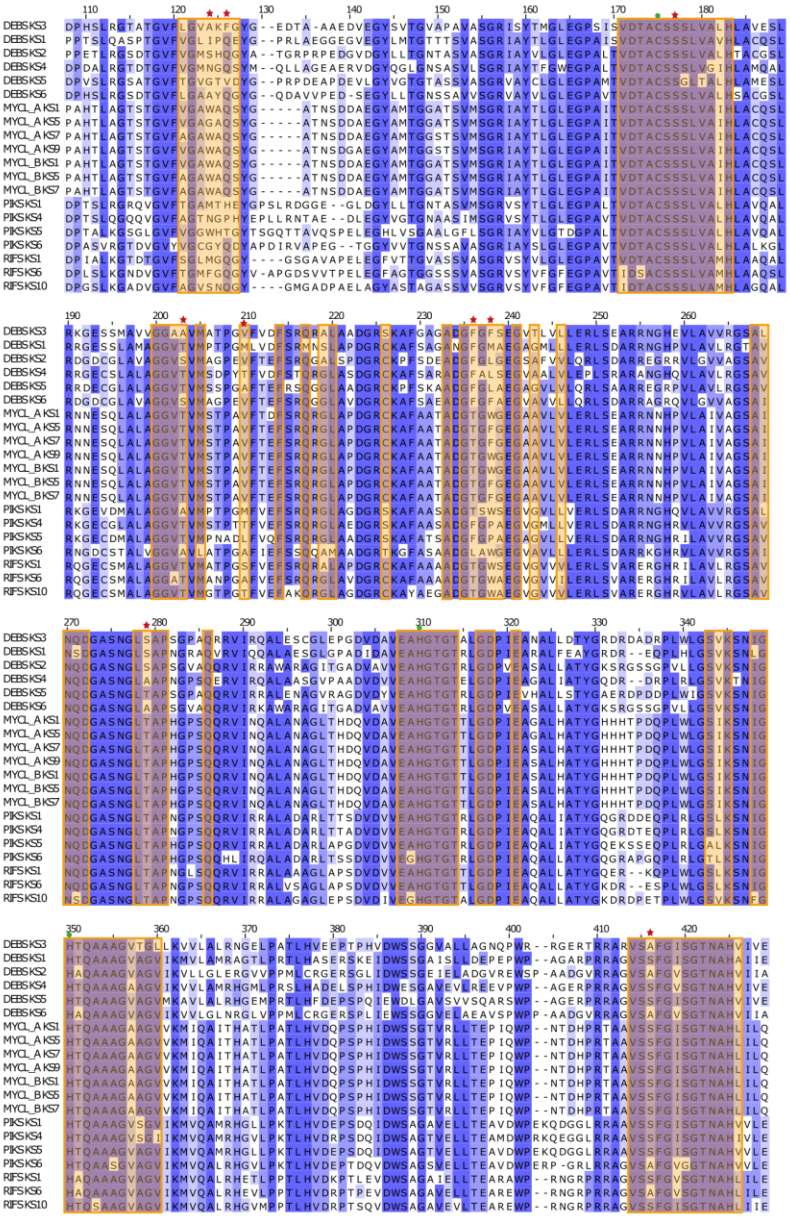

Figure S2. Sequence alignment of KS domains to identify residues for site-directed mutagenesis of DEBS KS6. Sequences were obtained from DEBS, mycolactone synthase (MYCL), PIKS, and RIFS. Residues within 12 Å of the active cysteine of DEBS KS6 (C175; Uniprot EryA3 Q03133 position 1661) are highlighted in orange. Green circles- catalytic triad, red stars- residues selected for multipoint mutagenesis (A124, Q126, S177, S203, V210, F236, F238, A279, A416). Nomenclature of selected residues according to homology model: A124, Q126, S174, S200, V207, F233, F235, A276, A412. Note that S177 (S174 in homology model) was accidently included in the diversified sequence space, although it is a conserved residue. According to the phylogenetic analysis implemented in FuncLib both Ala, and Gly can also be tolerated at this position (see Table S1).

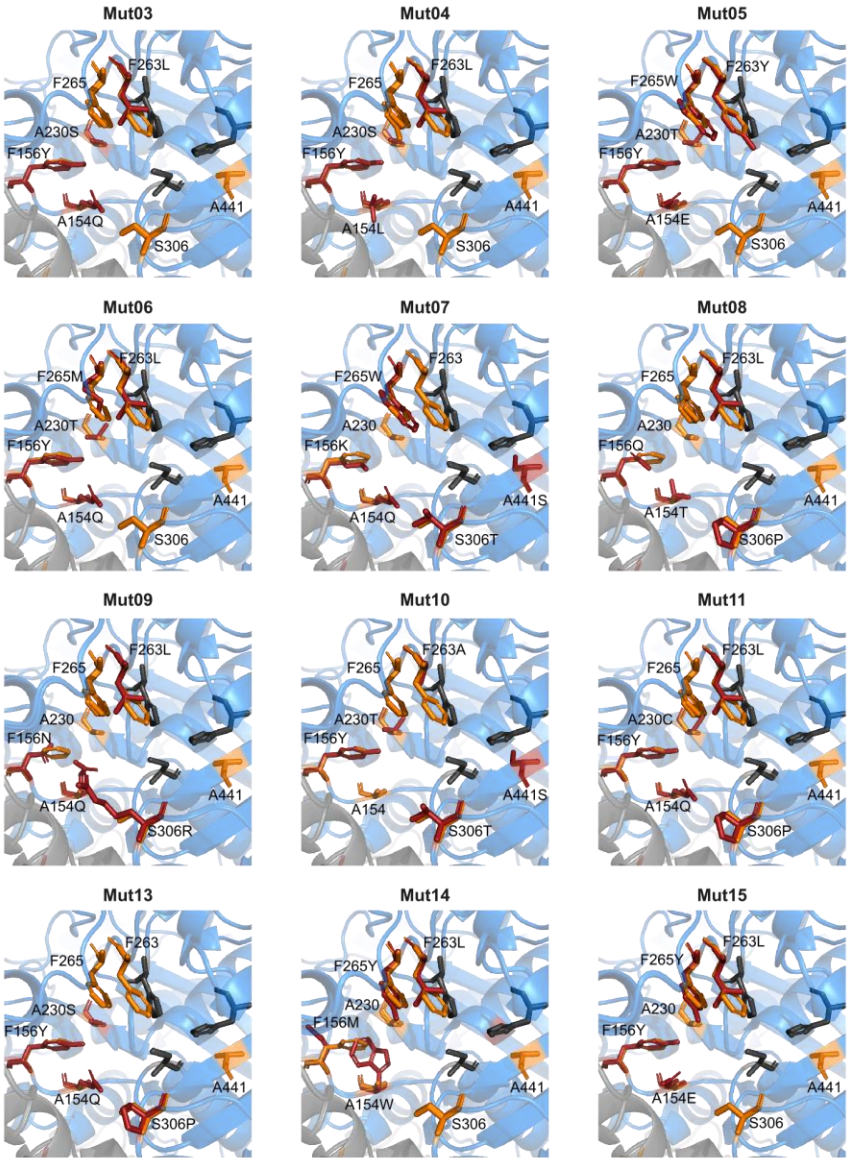

Figure S3. Structural alignment of wild-type and mutant KS3 binding sites. Alignment of wild-type KS3 (PDB 2QO3) and predicted structures of mutant KS3 proteins. Catalytic triad in gray, residues selected for mutagenesis in orange, and residues mutant in the respective design in red.

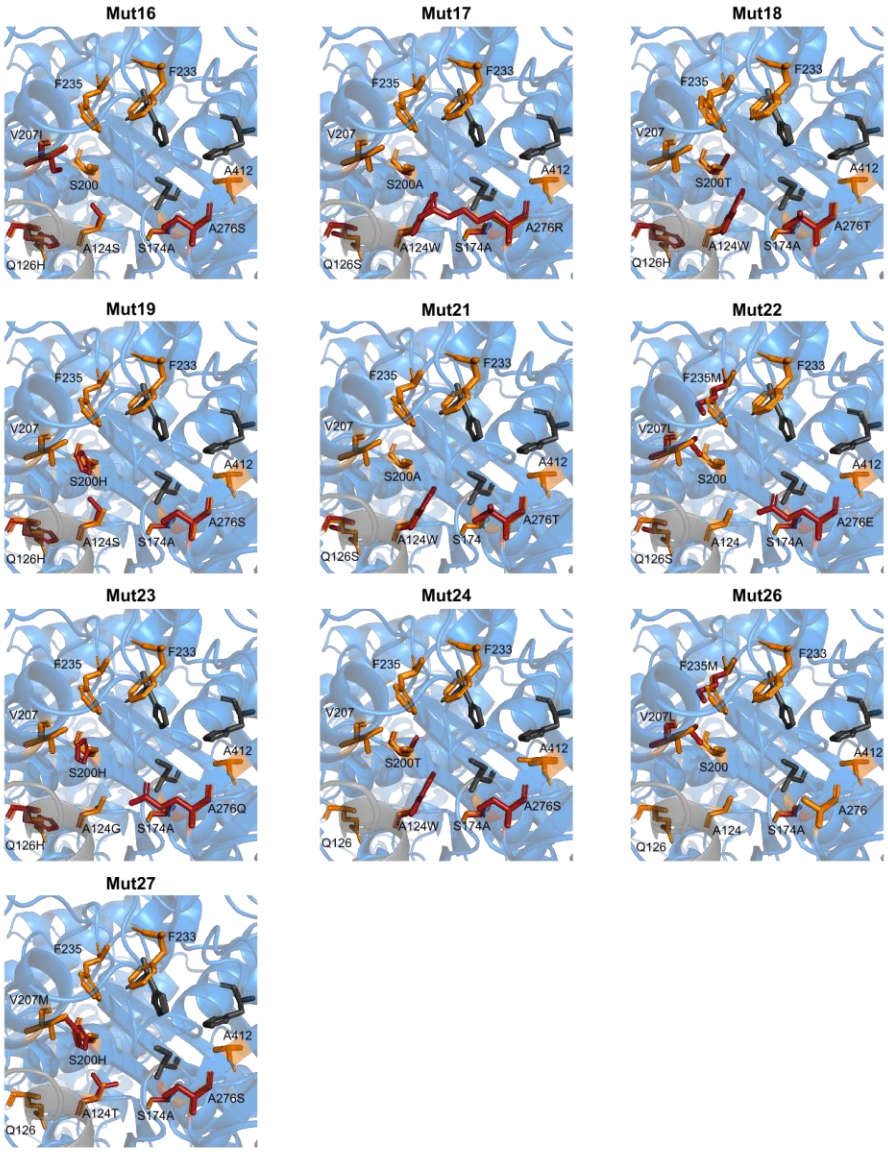

Figure S4. Structural alignment of wild-type and mutant KS6 binding sites. Alignment of wild-type KS6 (homology model) and predicted structures of mutant KS6 proteins. Catalytic triad in gray, residues selected for mutagenesis in orange, and residues mutant in the respective design in red.

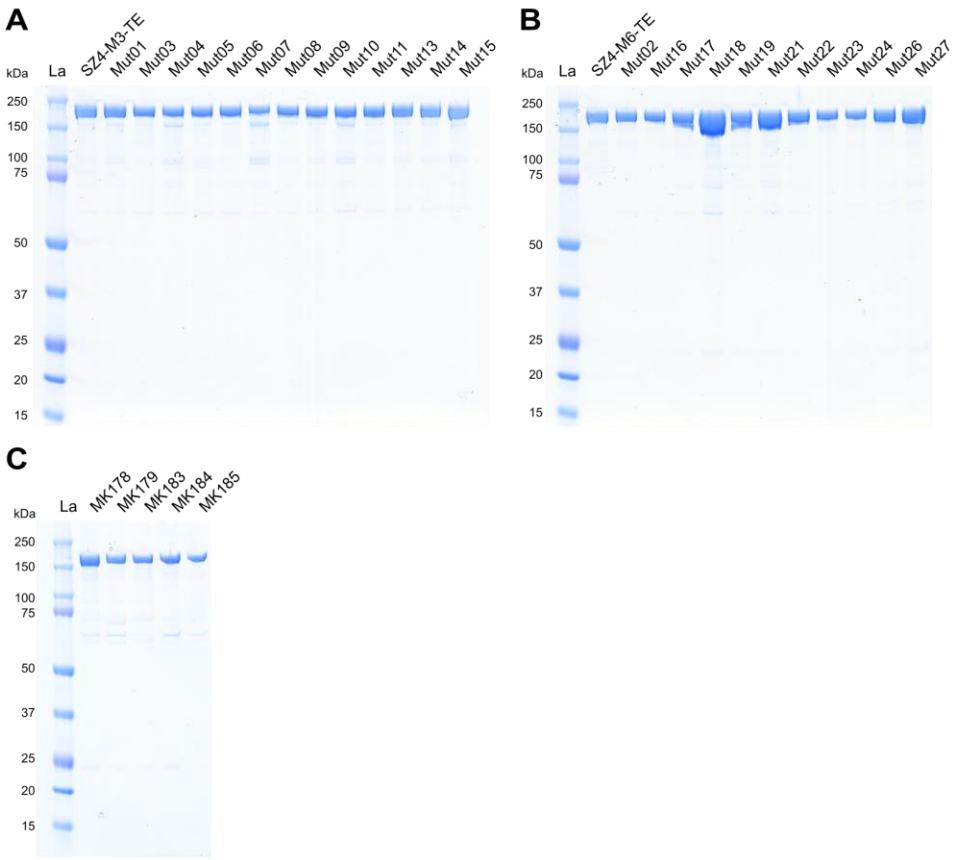

Figure S5. Purity of proteins used in this study. (A) SZ4-M3-TE and its mutants (MW 189 kDa), (B) SZ4-M6-TE and its mutants (MW 184 kDa), and (C) substrate-donating modules MK178 (Donor 4, 161 kDa), MK179 (Donor 6, 163 kDa), MK183 (Donor 2, 161 kDa), MK184 (Donor 3, 164 kDa), MK185 (Donor 5, 164 kDa).

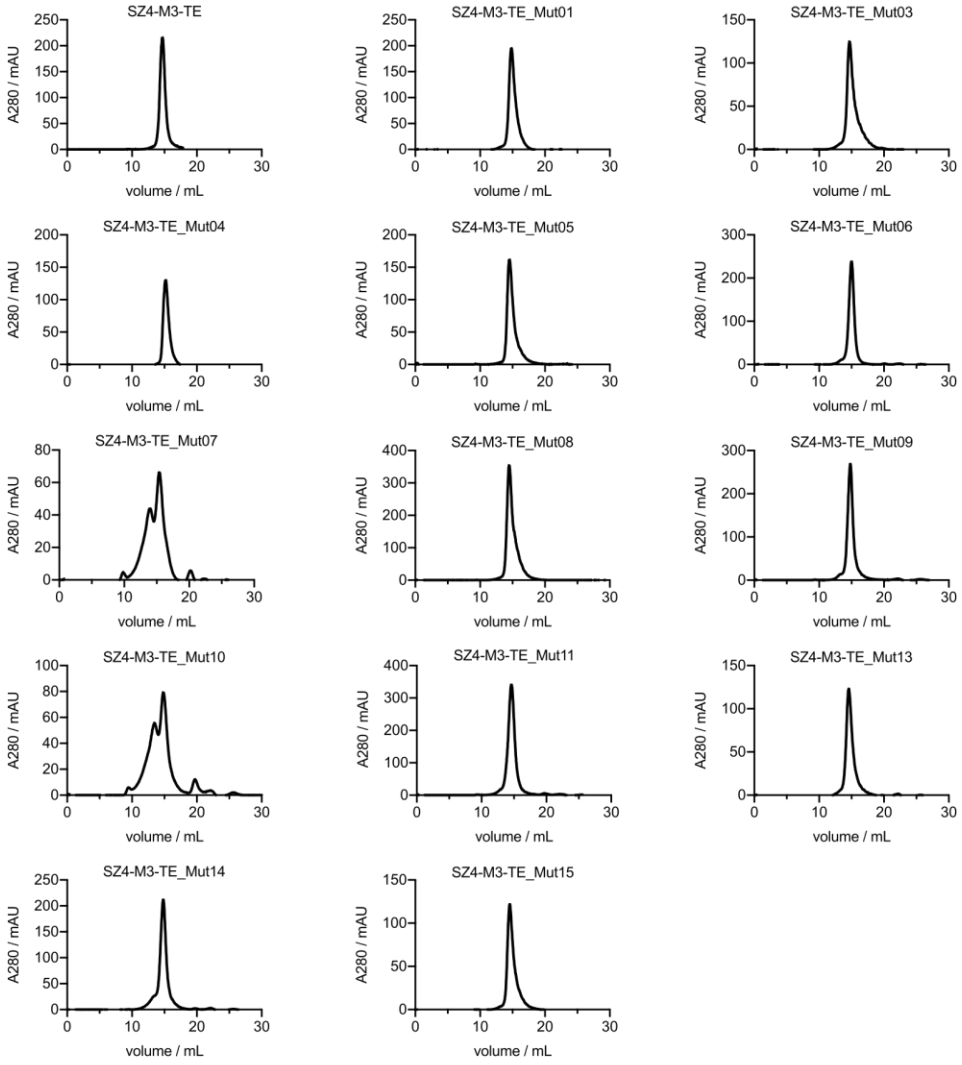

Figure S6. Analysis of proteins by SEC – M3 substrate-accepting modules. Wild-type M3-TE and its mutants analyzed by SEC. All proteins eluted in a predominately single peak from SEC.

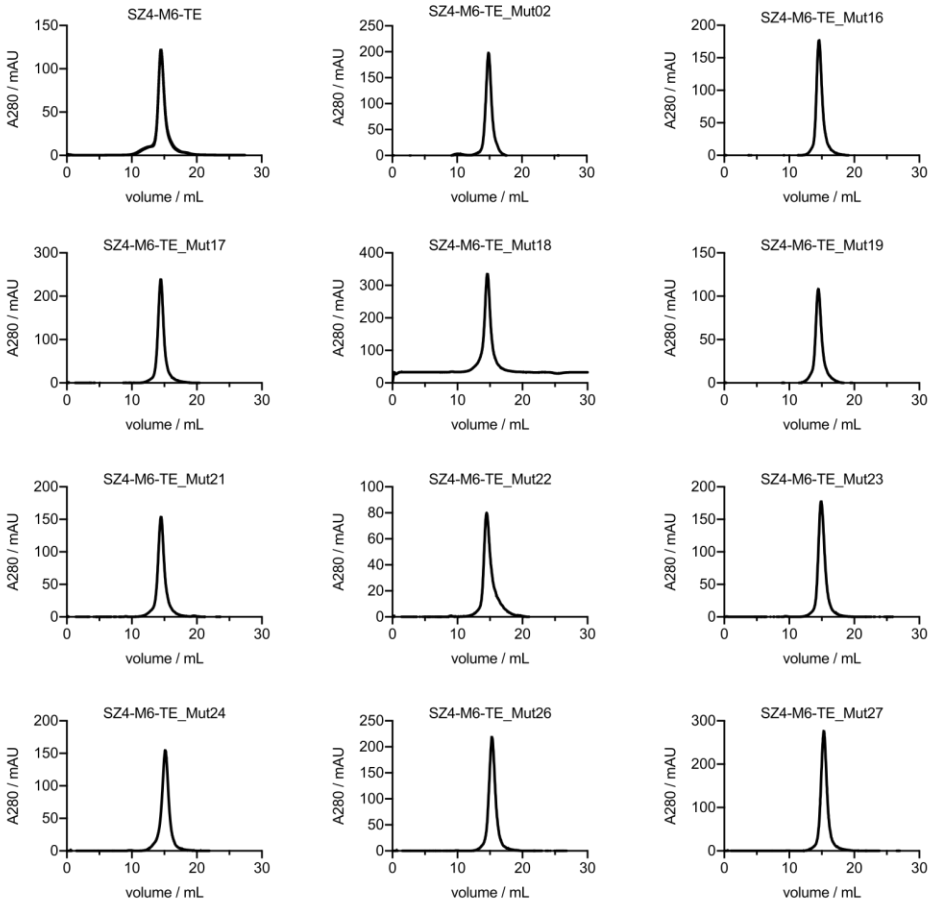

Figure S7. Analysis of proteins by SEC – M6 substrate-accepting modules. Wild-type M6-TE and its mutants analyzed by SEC. All proteins eluted in a predominately single peak from SEC.

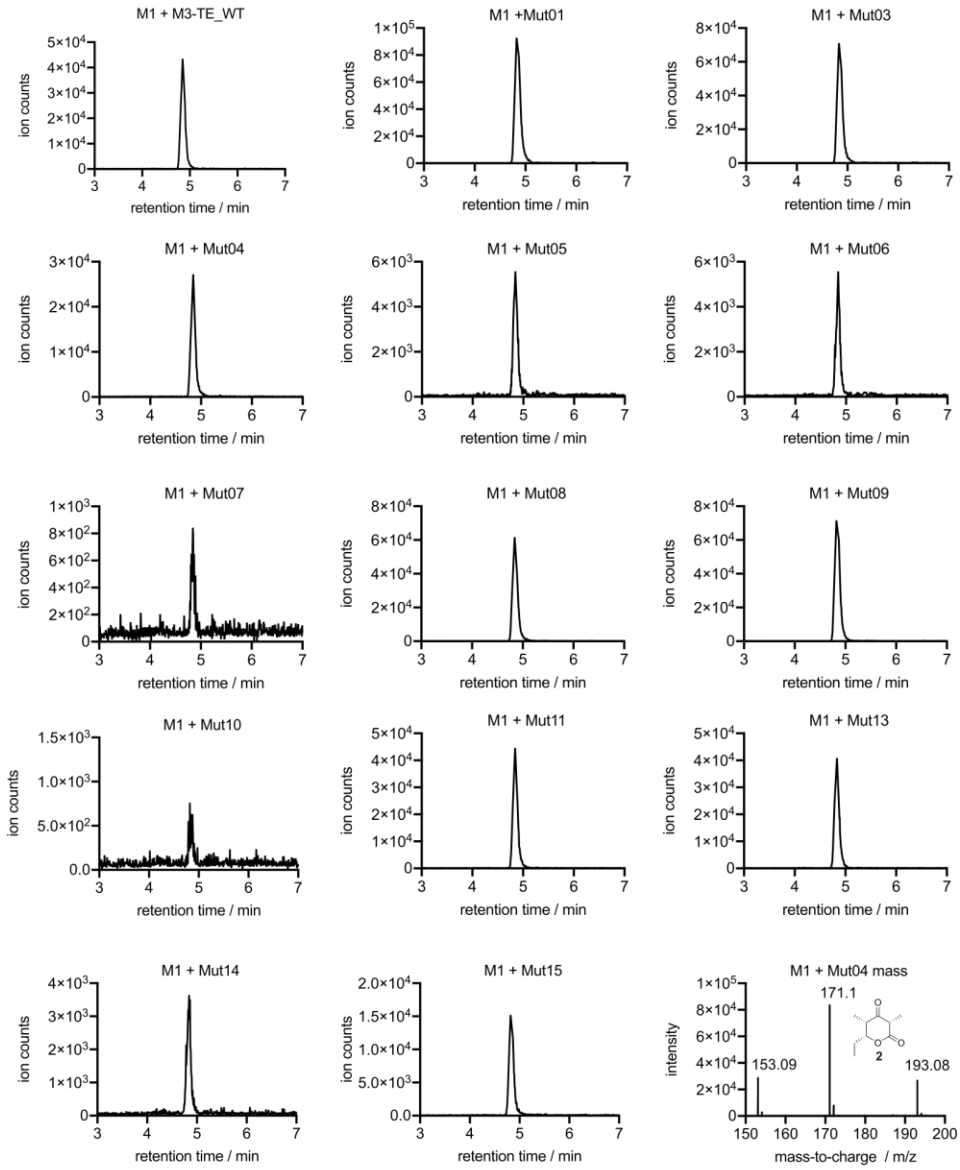

Figure S8. LC-MS analysis of unreduced TKL 2 produced by chimeric bimodular PKSs using (5)M1-SZ3 and SZ4-M3-TE. Unreduced TKL **2** ($C_9H_{14}O_3$, calculated MW = 170.22 g/mol) was detected in all reaction mixtures after overnight incubation. The extracted ion chromatograms were obtained by extraction of the $[M+H]^+$ species and one chromatogram per compound is shown as an example. Labeled peaks from left to right correspond to $[M+H-H2O]^+$, $[M+H]^+$, and $[M+Na]^+$ ions. TKL **2** eluted at 4.7 min.

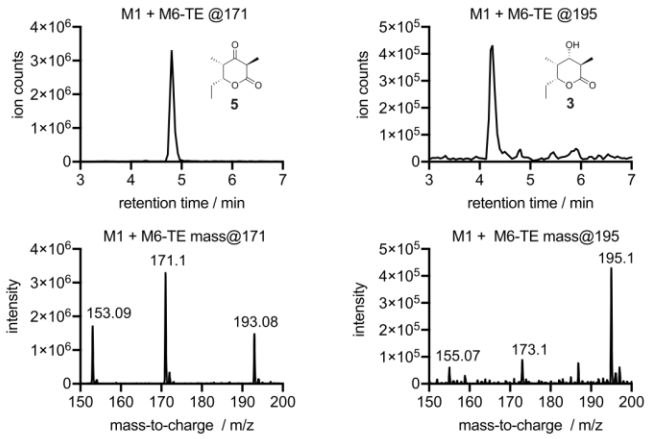

Figure S9. LC-MS analysis of reduced TKL 3 and unreduced TKL 5 produced by chimeric bimodular PKSs using (5)M1-SZ3 and SZ4-M6-TE. Reduced TKL **3** ($C_9H_{16}O_3$, calculated MW = 172.22 g/mol) and unreduced TKL **5** ($C_9H_{14}O_3$, calculated MW = 170.22 g/mol) were simultaneously detected in reaction mixtures shown in Figure 3B. The extracted ion chromatograms were obtained by extraction of the $[M+H]^+$ species (TKL **5**) or $[M+Na]^+$ species (TKL **3**) and one chromatogram per compound is shown as an example. Labeled peaks from left to right correspond to $[M+H-H2O]^+$, $[M+H]^+$, and $[M+Na]^+$ ions. TKL **5** eluted at 4.7 min and TKL **3** at 4.2 min.

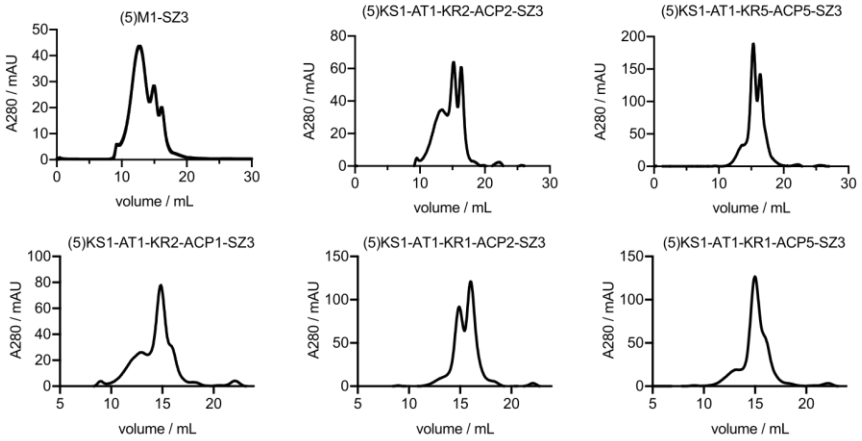

Figure S10. Analysis of substrate-donating modules by SEC. All proteins eluted in multiple oligomeric species from SEC.

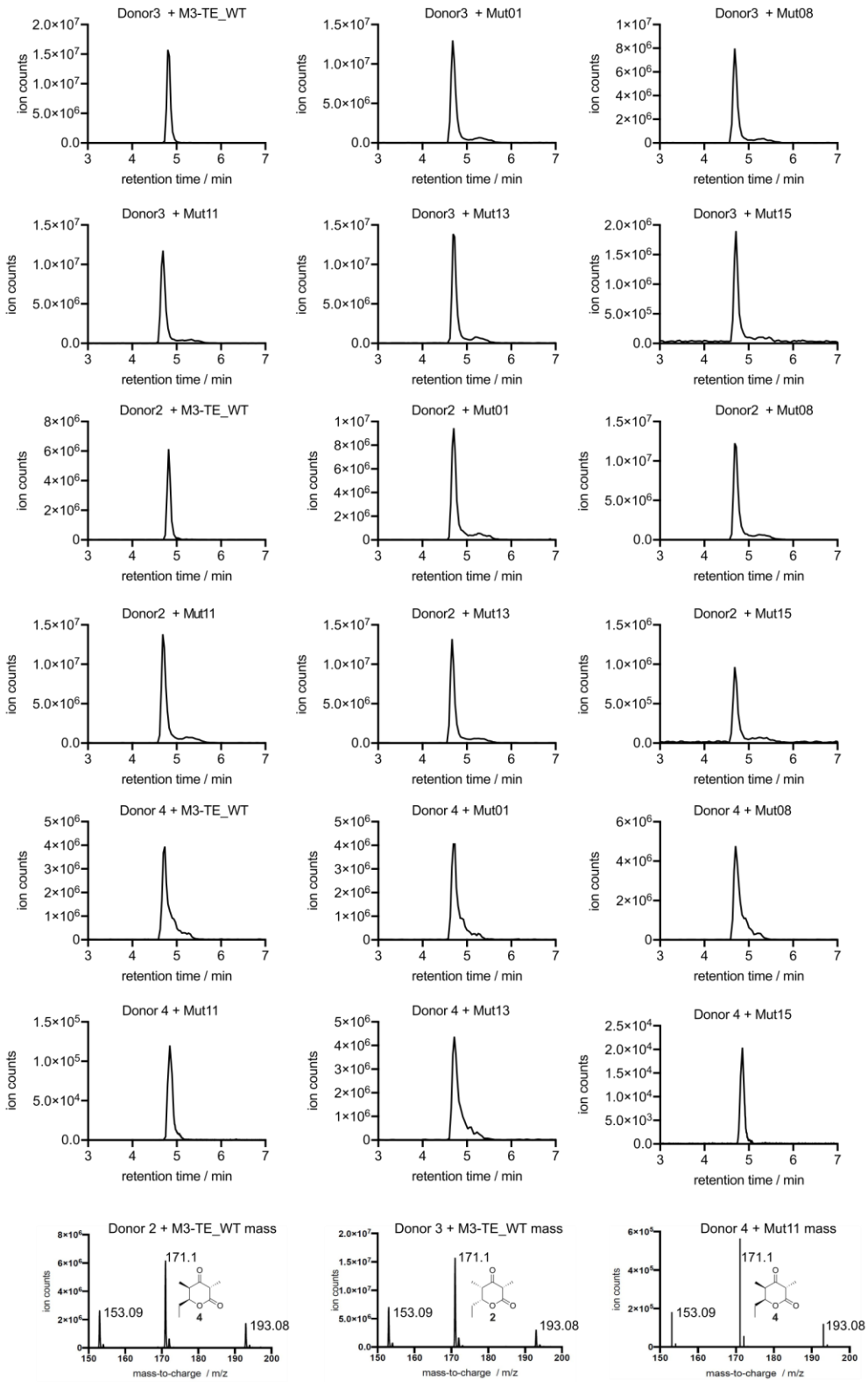

Figure S11. LC-MS analysis of unreduced TKL 2 (in case of Donor 3) and unreduced TKL 4 (in case of Donor 2 & 4) produced by chimeric bimodular PKSs using SZ4-M3-TE and different substrate-donating modules. Either (5)KS1-AT1-KR1-ACP2-SZ3 (Donor 3), (5)KS1-AT1-KR2-ACP1-SZ3 (Donor 2), or (5)KS1-AT1-KR2-ACP2-SZ3 (Donor 4) was used as the substrate-donating module**.** Unreduced TKL **2** or the diastereomeric

form unreduced TKL **4** ($C_9H_{14}O_3$, calculated MW = 170.22 g/mol) was detected in all reaction mixtures after overnight incubation. The extracted ion chromatograms were obtained by extraction of the $[M+H]^+$ species and one chromatogram per substrate-donating module is shown as an example. Labeled peaks from left to right correspond to $[M+H-H2O]^+$, $[M+H]^+$ and $[M+Na]^+$ ions.

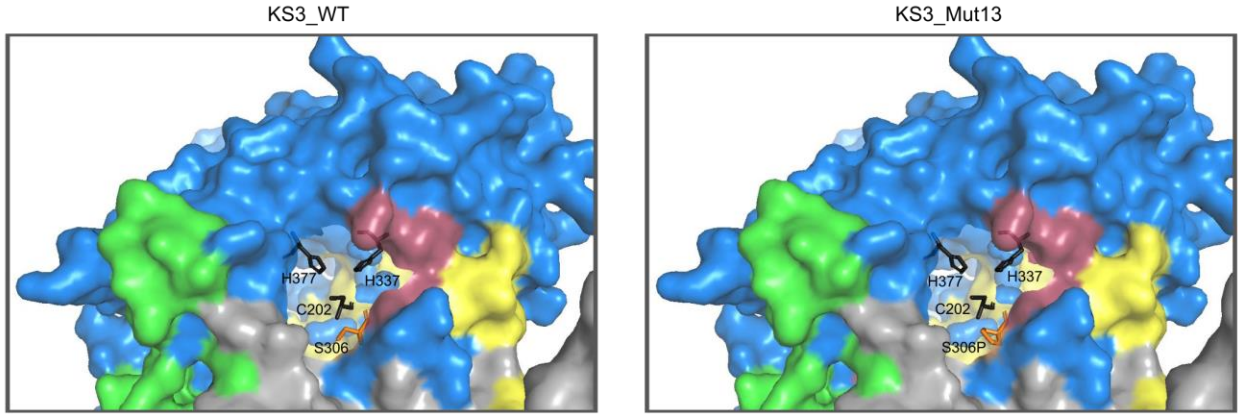

Figure S12. Structural organization of ACP docking interfaces and hydrophobic patch in DEBS KS3 and KS3_Mut13. The catalytic triad is depicted as black sticks. Position S306 which enlarged the conserved hydrophobic patch when mutated to S306P is depicted as orange stick. Docking interfaces of the upstream and intramodular ACP[1] are shown as yellow and green surfaces, respectively. The hydrophobic patch[2] is indicated as purple surface. Chain A and chain B of the KS3-AT3 didomain are colored in blue and grey, respectively.

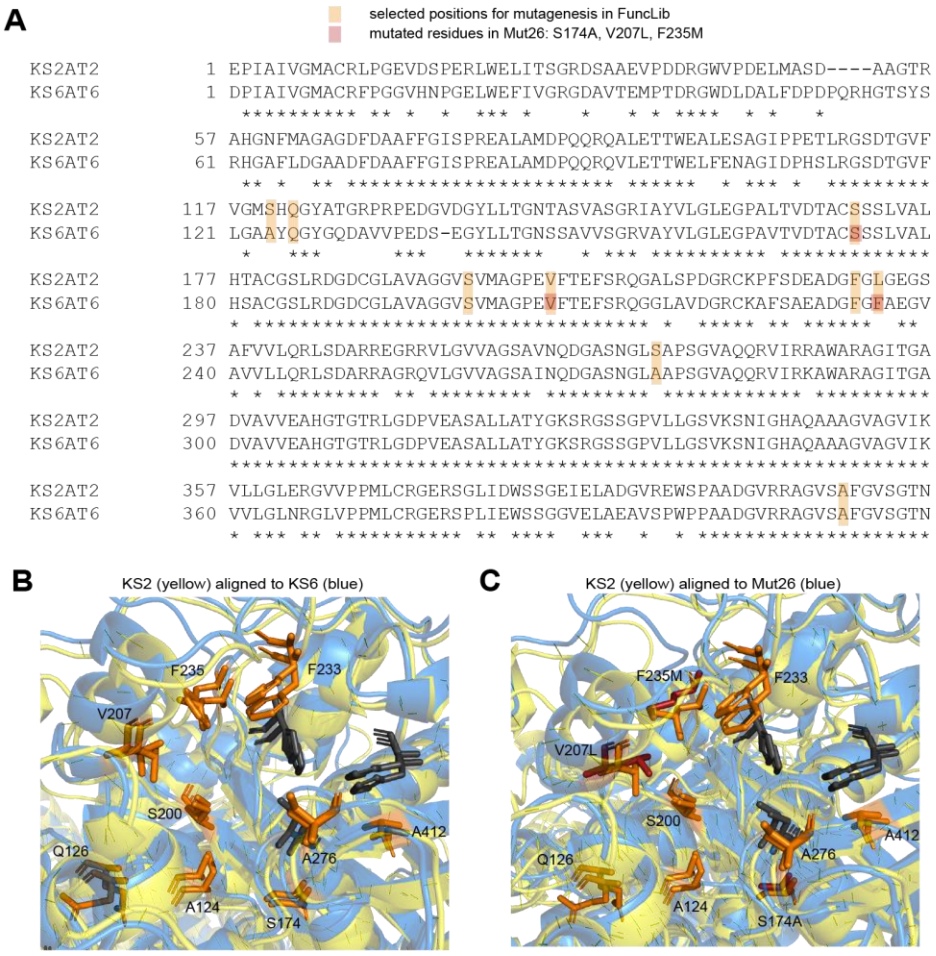

Figure S13. Sequence and structural alignment of DEBS KS2 and KS6. (A) Sequence alignment of KS2 and KS6. Residues selected for mutagenesis are highlighted in orange and those mutated in Mut26 in red. Structural alignment of KS2 to KS6 (B) and of KS2 to Mut26 (C). Catalytic triad in gray, residues selected for mutagenesis in orange, and positions mutated in Mut26 in red.

Supplementary Tables

Table S1. Sequence space of chosen binding site residues of KS3 and KS6 calculated by FuncLib

KS Domain	Position	Theoretical sequence space
KS3	A154	ADEGIKLMNQSTVW
	F156	FADEGHKMNQSTY
	A230	ACST
	F263	FALSY
	F265	FMWY
	S306	SAEGKMNPQRTV
	A441	AS
KS6	A124	AGNQSTW
	Q126	QAGHNST
	S174	SAG
	S200	SAHMNT
	V207	VACHILMNQST
	F233	FAS
	F235	FM
	A276	AEGKNQRST
	A412	ADEGS

Table S2. Yields of proteins used in this study. Typical yields are presented.

Construct	Protein	Yield/ mg/L of *E. coli* culture
MK149	SZ4-M3-TE	5.9
LB001	SZ4-M3-TE_Mut01	7.9
LB003	SZ4-M3-TE_Mut03	11.0
LB004	SZ4-M3-TE_Mut04	5.4
LB005	SZ4-M3-TE_Mut05	7.2
LB006	SZ4-M3-TE_Mut06	8.5
LB007	SZ4-M3-TE_Mut07	8.4
LB008	SZ4-M3-TE_Mut08	7.9
LB009	SZ4-M3-TE_Mut09	8.6
LB010	SZ4-M3-TE_Mut10	11.0
LB011	SZ4-M3-TE_Mut11	17.0
LB013	SZ4-M3-TE_Mut13	5.7
LB014	SZ4-M3-TE_Mut14	10.0
LB015	SZ4-M3-TE_Mut15	6.9
MK147	SZ4-M6-TE	6.3
LB002	SZ4-M6-TE_Mut02	5.5
LB016	SZ4-M6-TE_Mut16	9.1
LB017	SZ4-M6-TE_Mut17	11.0
LB018	SZ4-M6-TE_Mut18	11.0
LB019	SZ4-M6-TE_Mut19	8.2
LB021	SZ4-M6-TE_Mut21	9.6
LB022	SZ4-M6-TE_Mut22	5.1
LB023	SZ4-M6-TE_Mut23	6.2
LB024	SZ4-M6-TE_Mut24	6.5
LB026	SZ4-M6-TE_Mut26	7.2
LB027	SZ4-M6-TE_Mut27	7.9
BL12	LDD(4)	2.9
MK150	(5)M1-SZ3	4.7
MK148	SZ4-M2-TE	2.5
MK178	(5)KS1-AT1-KR2-ACP2-SZ3	4.1
MK179	(5)KS1-AT1-KR5-ACP5-SZ3	5.4
MK183	(5)KS1-AT1-KR2-ACP1-SZ3	2.7
MK184	(5)KS1-AT1-KR1-ACP2-SZ3	3.6
MK185	(5)KS1-AT1-KR1-ACP5-SZ3	6.5

Table S3. Melting temperatures of wild-type and mutant modules. N/A.- Not applicable due to higher oligomerization of protein in the sample (see Figure S6).

Construct	T_m / °C
MK147	41.7±0.3
MK149	41.5±0.0
LB001	40.0±0.0
LB002	41.5±0.0
LB003	41.7±0.3
LB004	40.7±0.3
LB005	41.5±0.0
LB006	42.5±0.0
LB007	N/A
LB008	41.2±0.3
LB009	43.7±0.3
LB010	N/A
LB011	41.5±0.7
LB013	41.2±0.3
LB014	46.0±0.0
LB015	41.7±0.3
LB016	41.2±0.3
LB017	36.7±1.0
LB018	40.7±0.3
LB019	42.7±0.3
LB021	41.0±0.0
LB022	40.5±0.0
LB023	38.5±0.0
LB024	40.0±0.7
LB026	40.2±0.3
LB027	42.0±0.7

Table S4. Distribution of reduced and unreduced triketide lactone products in bimodular chimeric PKSs using LDD(4), (5)M1-SZ3 and the listed substrate-accepting modules. Peak areas were calculated from LC-MS measurements after the reaction time of 10 min. Peaks were obtained by searching for the respective $[M+H]^+$-species. Reduced TKL **3** eluted at 4.2 min and unreduced TKL **5** at 4.7 min.

acceptor module	red. TKL, peak area	unred. TKL, peak area	red. TKL: unred. TKL
M2-TE	$2.3x10^7$	$7.1x10^6$	3.2 : 1
M6-TE_WT	$5.9x10^6$	$1.7x10^8$	1 : 28.8
M6-TE_Mut02	$4.2x10^6$	$1.1x10^8$	1 : 26.1
M6-TE_Mut16	$6.3x10^7$	$2.0x10^8$	1 : 3.1
M6-TE_Mut17	$2.2x10^6$	$1.0x10^8$	1 : 45.4
M6-TE_Mut18	$4.2x10^6$	$1.3x10^8$	1 : 30.9
M6-TE_Mut19	$1.8x10^6$	$5.4x10^7$	1 : 30.0
M6-TE_Mut21	$4.5x10^6$	$1.2x10^8$	1 : 26.6
M6-TE_Mut22	$2.1x10^6$	$3.9x10^7$	1 : 18.5
M6-TE_Mut23	$2.4x10^6$	$6.4x10^7$	1 : 26.6
M6-TE_Mut24	$6.9x10^6$	$2.3x10^8$	1 : 33.3
M6-TE_Mut26	$1.3x10^7$	$5.1x10^8$	1 : 39.2
M6-TE_Mut27	$5.1x10^5$	$3.0x10^7$	1 : 58.8

Table S5. Peak area of reduced and unreduced triketide lactone products in bimodular chimeric PKSs using SZ4-M6-TE or Mut26 in the presence of different substrate-donating modules. The identity of the substrate on the upstream ACP is indicated. Peak areas were calculated from LC-MS measurements after a reaction time of 10 min. Peaks were obtained by searching for the respective $[M+H]^+$-species. Reduced TKL **3** & **6** eluted at 4.2 min and unreduced TKL **5** & **7** at 4.7 min. Note that the ratio does not reflect the absolute amount of TKL product in the sample as no TKL standard was available to capture different ionization behaviors of the two compounds.

Acceptor module	Donor module (Abbreviation and full name)	Substrate bound to upstream ACP	red. TKL, peak area	unred. TKL, peak area
M6-TE	1, M1	NDK	$5.9x10^6$	$1.7x10^8$
	5, KS1-AT1-KR1-ACP5	NDK	none	$5.8x10^7$
	2, KS1-AT1-KR2-ACP1	EDK	$1.1x10^7$	$6.4x10^6$
	6, KS1-AT1-KR5-ACP5	EDK	$3.6x10^6$	$2.6x10^6$
M6-TE_Mut26	1, M1	NDK	$1.3x10^7$	$5.1x10^8$
	5, KS1-AT1-KR1-ACP5	NDK	$3.1x10^6$	$9.3x10^7$
	2, KS1-AT1-KR2-ACP1	EDK	$1.1x10^7$	$1.5x10^6$
	6, KS1-AT1-KR5-ACP5	EDK	$1.8x10^7$	$2.1x10^6$

Table S6. Plasmids used in this study and their origin

Plasmid	Origin
pBL12_LDD(4)_pET28_kan	Lowry *et al.*[3]
pMK147_SZ4-M6-TE-H6_pET22_carb	Klaus *et al.*[4]
pMK148_SZ4-M2-TE-H6_pET22_carb	Klaus *et al* [4]
pMK149_SZ4-M3-TE-H6_pET22_carb	Klaus *et al.*[4]
pMK150_(5)M1-SZ3-H6_pET22b_carb	Klaus *et al.*[4]
pMK178_(5)KS1-AT1-KR2-ACP2-SZ3-H6_pET22b_carb	this study
pMK179_(5)KS1-AT1-KR5-ACP5-SZ3-H6_pET22b_carb	this study
pMK183_(5)KS1-AT1-KR2-ACP1-SZ3-H6_pET22b_carb	this study
pMK184_(5)KS1-AT1-KR1-ACP2-SZ3-H6_pET22b_carb	this study
pMK185_(5)KS1-AT1-KR1-ACP5-SZ3-H6_pET22b_carb	this study
pLB001_SZ4-M3-TE-H6_A189W_pET22_carb	this study
pLB002_SZ4-M6-TE-H6_A189W_pET22_carb	this study
pLB003_SZ4-M3-TE-H6_A189Q_F191Y_A265S_F298L_pET22_carb	this study
pLB004_SZ4-M3-TE-H6_A189L_F191Y_A265S_F298L_F300Y_pET22_carb	this study
pLB005_SZ4-M3-TE-H6_A19E_F191Y_A165T_F298Y_F300W_pET22_carb	this study
pLB006_SZ4-M3-TE-H6_A189Q_F191Y_A265T_F298L_F300M_pET22_carb	this study
pLB007_SZ4-M3-TE-H6_A189Q_F191K_F298W_S341T_A476S_pET22_carb	this study
pLB008_SZ4-M3-TE-H6_A189T_F191Q_F298L_S306P_pET22_carb	this study
pLB009_SZ4-M3-TE-H6_A189Q_F191N_F298L_S341R_pET22_carb	this study
pLB010_SZ4-M3-TE-H6_F191Y_A265T_F298A_S341T_A476S_pET22_carb	this study
pLB011_SZ4-M3-TE-H6_A189Q_F191Y_A265C_F298L_S341P_pET22_carb	this study
pLB013_SZ4-M3-TE-H6_A189Q_F156Y_A265S_S341P_pET22_carb	this study
pLB014_SZ4-M3-TE-H6_A189W_F191M_F298L_F300Y_pET22_carb	this study
pLB015_SZ4-M3-TE-H6_A189E_F191Y_F298L_F300Y_pET22_carb	this study
pLB016_SZ4-M6-TE-H6_A189S_Q191H_S239A_V272I_A341S_pET22_carb	this study
pLB017_SZ4-M6-TE-H6_A189W_Q191S_S239A_S265A_A341R_pET22_carb	this study
pLB018_SZ4-M6-TE-H6_A189W_Q191H_S239A_S265T_A341T_pET22_carb	this study
pLB019_SZ4-M6-TE-H6_A189S_Q191H_S239A_S265H_A341S_pET22_carb	this study
pLB021_SZ4-M6-TE-H6_A189W_Q191S_S265A_A341T_pET22_carb	this study
pLB022_SZ4-M6-TE-H6_Q191S_S239A_V272L_F300M_A341E_pET22_carb	this study
pLB023_SZ4-M6-TE-H6_A189G_Q191H_S239A_S265H_A341Q_pET22_carb	this study
pLB024_SZ4-M6-TE-H6_A189W_S239A_S265T_A341S_A477S_pET22_carb	this study
pLB026_SZ4-M6-TE-H6_S239A_V272L_F300M_pET22_carb	this study
pLB027_SZ4-M6-TE-H6_A189T_S239A_S265H_V272M_A341S_pET22_carb	this study

Table S7. Cloning strategy of plasmids generated in this study. Individual fragments were generated by overlap extension PCR, conventional PCR or ordered as gBlocks and assembled via In-Fusion cloning. .Single point mutations were introduced via QuickChange site-directed mutagenesis. See Table S8 for primer used to generate individual fragments and Table S9 for sequences of gBlocks.

Plasmid	Cloning Method	Fragments	Primer Name	Primer Sequence 5'-3'	Template
pLB003	In-Fusion	1 + 7	P-LB003	CTCGCCGTAGCCGAACTTCcaCACTCCGAGGAAGACGCC	pMK149
			P-LB050	TCCGCTCGCCGCGCC	
		5 + 3	P-LB051	GCGCGAGGGCGGTGAG	pMK149
			P-LB009	CGGGGTCGCCATCACCGaGGCACCGCCGACGACC	
		pLB003V	P-LB052	CTCACCGCCCTCGCGC	pMK149
			P-LB053	GGCGCGGCGAGCGGA	
pLB004	In-Fusion	4 + 6	P-LB051	GCGCGAGGGCGGTGAG	pMK149
			P-LB009	CGGGGTCGCCATCACCGaGGCACCGCCGACGACC	
		2 + 8	P-LB008	GGTCGTCGGCGGTGCCtCGGTGATGGCGACCCCG	pMK149
			P-LB050	TCCGCTCGCCGCGCC	
		pLB003V	P-LB052	CTCACCGCCCTCGCGC	pMK149
			P-LB053	GGCGCGGCGAGCGGA	
pLB005	In-Fusion	11 + 17 + 21 + 35	P-LB050	TCCGCTCGCCGCGCC	pMK149
			P-LB051	GCGCGAGGGCGGTGAG	
		pLB003V	P-LB052	CTCACCGCCCTCGCGC	pMK149
			P-LB053	GGCGCGGCGAGCGGA	
pLB006	In-Fusion	22 + 18	P-LB051	GCGCGAGGGCGGTGAG	pMK149
			P-LB019	CGGGGTCGCCATCACCGtGGCACCGCCGACGACC	
		12 + 36	P-LB018	GGTCGTCGGCGGTGCCaCGGTGATGGCGACCCCG	pMK149
			P-LB050	TCCGCTCGCCGCGCC	
		pLB003V	P-LB052	CTCACCGCCCTCGCGC	pMK149
			P-LB053	GGCGCGGCGAGCGGA	

Plasmid	Cloning Method	Fragments	Primer Name	Primer Sequence 5'-3'	Template
pLB007	In-Fusion	pLB007_gblock			
		pLB007V	P-LB062opt	TCCGAGGAATACGCCGGTCGCGGTACCGCGC	pMK149
			P-LB063opt	TTCGGTATTAGCGGGACGAATGCGCACGTGATCGTC	
pLB008	In-Fusion	25 + 27	P-LB051	GCGCGAGGGCGGTGAG	pMK149
			P-LB011	GACGCCTTCGGAGAAGCCtAACCCGTCGGCGCCGG	
		13 + 32	P-LB010	CCGGCGCCGACGGGTTaGGCTTCTCCGAAGGCGTC	pMK149
			P-LB050	TCCGCTCGCCGCGCC	
		pLB003V	P-LB052	CTCACCGCCCTCGCGC	pMK149
			P-LB053	GGCGCGGCGAGCGGA	
pLB009	In-Fusion	pLB009gblock			
		pLB009V	P-MK550	CCCCAAGAATACGCCGGTCGCGGTACCGCGC	pMK149
			P-MK551	CGCGCACCCTCGGGGCCCGCGCAGCGCAGGG	
pLB010	In-Fusion	pLB010gblock			
		pLB010V	P-MK552	CGCCACACCAAGGAAGACGCCGGTCGCGGTACC	pMK149
			P-MK553	TTCGGGATCTCTGGGACGAATGCGCACGTGATCGTC	
pLB011	In-Fusion	pLB011gblock			
		pLB011V	P-MK554	TCCTAAGAAGACGCCGGTCGCGGTACCGCGC	pMK149
			P-MK555	CCTGCCCCTTCAGGGCCCGCGCAGCGCAGG	
pLB013	In-Fusion	16 + 32	P-LB008	GGTCGTCGGCGGTGCCtCGGTGATGGCGACCCCG	pMK149
			P-LB050	TCCGCTCGCCGCGCC	
		22 + 19	P-LB050	TCCGCTCGCCGCGCC	pMK149
			P-LB009	CGGGGTCGCCATCACCGaGGCACCGCCGACGACC	
		pLB003V	P-LB052	CTCACCGCCCTCGCGC	pMK149
			P-LB053	GGCGCGGCGAGCGGA	

Plasmid	Cloning Method	Fragments	Primer Name	Primer Sequence 5'-3'	Template
			P-LB015	GGGTGACGCCTTCGGAGtAGCCtAACCCGTCGGCGCCGG	
pLB015	In-Fusion	21			
		30 + 37	P-LB016	GCGTCTTCCTCGGAGTGGaGAAGTaCGGCTACGGCGAGGAC	pMK149
			P-LB050	TCCGCTCGCCGCGCC	
		pLB003V	P-LB052	CTCACCGCCCTCGCGC	pMK149
			P-LB053	GGCGCGGCGAGCGGA	
pLB016	In-Fusion	pLB016gblock			
		pLB016V	P-LB073	GGCCCCCAAGAAGACGCCGGTGTCGC	pMK147
			P-LB074	GCGCCTTCGGGCGTCGCCCAGCAGC	
pLB017	In-Fusion	pLB017gblock			
		pLB016V	P-LB073	GGCCCCCAAGAAGACGCCGGTGTCGC	pMK147
			P-LB074	GCGCCTTCGGGCGTCGCCCAGCAGC	
pLB018	In-Fusion	pLB018gblock			
		pLB016V	P-LB073	GGCCCCCAAGAAGACGCCGGTGTCGC	pMK147
			P-LB074	GCGCCTTCGGGCGTCGCCCAGCAGC	
pLB019	In-Fusion	pLB019gblock			
		pLB016V	P-LB073	GGCCCCCAAGAAGACGCCGGTGTCGC	pMK147
			P-LB074	GCGCCTTCGGGCGTCGCCCAGCAGC	
pLB021	In-Fusion	pLB021gblock			
		pLB016V	P-LB073	GGCCCCCAAGAAGACGCCGGTGTCGC	pMK147
			P-LB074	GCGCCTTCGGGCGTCGCCCAGCAGC	
pLB022	In-Fusion	pLB022gblock			
		pLB016V	P-LB073	GGCCCCCAAGAAGACGCCGGTGTCGC	pMK147
			P-LB074	GCGCCTTCGGGCGTCGCCCAGCAGC	

Plasmid	Cloning Method	Fragments	Primer Name	Primer Sequence 5'-3'	Template
pLB023	In-Fusion	pLB023gblock			
		pLB016V	P-LB073	GGCCCCCAAGAAGACGCCGGTGTCGC	pMK147
			P-LB074	GCGCCTTCGGGCGTCGCCCAGCAGC	
pLB024	In-Fusion	pLB024gblock			
		pLB024V	P-LB073	GGCCCCCAAGAAGACGCCGGTGTCGC	pMK147
			P-LB074	GCGCCTTCGGGCGTCGCCCAGCAGC	
pLB026	In-Fusion	pLB026gblock			
		pLB016V	P-LB073	GGCCCCCAAGAAGACGCCGGTGTCGC	pMK147
			P-LB074	GCGCCTTCGGGCGTCGCCCAGCAGC	
pLB027	In-Fusion	pLB027glbock			
		pLB016V	P-LB073	GGCCCCCAAGAAGACGCCGGTGTCGC	pMK147
			P-LB074	GCGCCTTCGGGCGTCGCCCAGCAGC	
pMK178	In-Fusion	pMK178_I	P-MK517	GAGCGCGTCTGGCTCCTGCCGGACCGCACCAC	pMK175
			P-MK518	GCCACCGGATCCGCCGCCGAGCTCACTAGTGAGG	
		pMK178_V	P-MK312	GGATCCGCCACCGGATCCGCCTTCAGCAACATCGTTCTCCAATCTG	pMK111
			P-MK483	GGCGGATCCGGTGGCGGA	
pMK179	In-Fusion	pMK179_I	P-MK519	GAGCGCGTCTGGCTCCCCATCCCCACCGGCG	pMK168
			P-MK520	GCCACCGGATCCGCCGACGAGCCGCTCCAGGTA	
		pMK179_V	P-MK312	GGATCCGCCACCGGATCCGCCTTCAGCAACATCGTTCTCCAATCTG	pMK111
			P-MK483	GGCGGATCCGGTGGCGGA	
pMK183	In-Fusion	pMK183_I	P-MK260	GAAGGAGATATACATATGAGCGGTGACAACGGCATGACCGAGGAAAAG	pMK178

Plasmid	Cloning Method	Fragments	Primer Name	Primer Sequence 5'-3'	Template
			P-MK560	CCGGTCGCGCAGGCTC	
		pMK183_V	P-MK559	ACGGAGAGCCTGCGCGACCGGCTGGCGTCGCTGCCCG	pMK150
			P-MK239	CATATGTATATCTCCTTCTTAAAGTTAAACAAAATTA	
pMK184	In-Fusion	pMK184_I	P-MK260	GAAGGAGATATACATATGAGCGGTGACAACGGCATGACCGAGGAAAAG	pMK150
			P-MK562	CGCGCCCACCCGCGG	
		pMK184_V	P-MK239	CATATGTATATCTCCTTCTTAAAGTTAAACAAAATTA	pMK178
			P-MK561	GCCGAACCGCGGGTGGGCGCGCTGGCGGGTCTGCCGC	
pMK185	In-Fusion	pMK185_I	P-MK260	GAAGGAGATATACATATGAGCGGTGACAACGGCATGACCGAGGAAAAG	pMK150
			P-MK562	CGCGCCCACCCGCGG	
		pMK185_V	P-MK563	GCCGAACCGCGGGTGGGCGCGCTCGCGGCGCTGTCGAC	pMK179
			P-MK239	CATATGTATATCTCCTTCTTAAAGTTAAACAAAATTA	
pLB001	QuickChange	-	P-LB002	GGCGTCTTCCTCGGAGTGtgGAAGTTCGGCTACGGCGAG	pMK149
			P-LB003	CTCGCCGTAGCCGAACTTCcaCACTCCGAGGAAGACGCC	
pLB002	QuickChange	-	P-LB004	CGTCTTCCTCGGCGCCtgGTACCAGGGCTACGGCC	pMK147
			P-LB005	GGCCGTAGCCCTGGTACcaGGCGCCGAGGAAGACG	

Table S8. Cloning strategy of fragments used in In-Fusion cloning and primer sequences

Fragment	Primer Name	Primer Sequence 5'-3'	Template	Used to generate
1	P-LB008	GGTCGTCGGCGGTGCCtCGGTGATGGCGACCCCG	pMK149	pLB003
	P-LB011	GACGCCTTCGGAGAAGCCtAACCCGTCGGCGCCGG		
2	P-LB008	GGTCGTCGGCGGTGCCtCGGTGATGGCGACCCCG	pMK149	pLB004
	P-LB015	GGGTGACGCCTTCGGAGtAGCCtAACCCGTCGGCGCCGG		
3	P-LB006	GGCGTCTTCCTCGGAGTGcaGAAGTaCGGCTACGGCGAGGAC	pMK149	pLB003
	P-LB009	CGGGGTCGCCATCACCGaGGCACCGCCGACGACC		
4	P-LB012	GGCGTCTTCCTCGGAGTGctGAAGTaCGGCTACGGCGAGGAC	pMK149	pLB004
	P-LB009	CGGGGTCGCCATCACCGaGGCACCGCCGACGACC		
5	P-LB051	GCGCGAGGGCGGTGAG	pMK149	pLB003
	P-LB007	GTCCTCGCCGTAGCCGtACTTCtgCACTCCGAGGAAGACGCC		
6	P-LB051	GCGCGAGGGCGGTGAG	pMK149	pLB004
	P-LB013	GTCCTCGCCGTAGCCGtACTTCagCACTCCGAGGAAGACGCC		
7	P-LB010	CCGGCGCCGACGGGTTaGGCTTCTCCGAAGGCGTC	pMK149	pLB003
	P-LB050	TCCGCTCGCCGCGCC		
8	P-LB014	CCGGCGCCGACGGGTTaGGCTaCTCCGAAGGCGTCACCC	pMK149	pLB004
	P-LB050	TCCGCTCGCCGCGCC		
11	P-LB018	GGTCGTCGGCGGTGCCaCGGTGATGGCGACCCCG	pMK149	pLB005
	P-LB021	CAGGGTGACGCCTTCGGAccAGCCGtACCCGTCGGCGCCGG		
12	P-LB018	GGTCGTCGGCGGTGCCaCGGTGATGGCGACCCCG	pMK149	pLB006
	P-LB023	CAGGGTGACGCCTTCGGAcAtGCCtAACCCGTCGGCGCCGG		
13	P-LB010	CCGGCGCCGACGGGTTaGGCTTCTCCGAAGGCGTC	pMK149	pLB008
	P-LB035	CCCGCTCGGCGCGGgAAGCCCGTTGCTGGCCC		
16	P-LB008	GGTCGTCGGCGGTGCCtCGGTGATGGCGACCCCG	pMK149	pLB013
	P-LB035	CCCGCTCGGCGCGGgAAGCCCGTTGCTGGCCC		
17	P-LB016	GCGTCTTCCTCGGAGTGGaGAAGTaCGGCTACGGCGAGGAC	pMK149	pLB005
	P-LB019	CGGGGTCGCCATCACCGtGGCACCGCCGACGACC		

Fragment	Primer Name	Primer Sequence 5'-3'	Template	Used to generate
18	P-LB006	GGCGTCTTCCTCGGAGTGcaGAAGTaCGGCTACGGCGAGGAC	pMK149	pLB006
	P-LB019	CGGGGTCGCCATCACCGtGGCACCGCCGACGACC		
19	P-LB006	GGCGTCTTCCTCGGAGTGcaGAAGTaCGGCTACGGCGAGGAC	pMK149	pLB013
	P-LB009	CGGGGTCGCCATCACCGaGGCACCGCCGACGACC		
21	P-LB051	GCGCGAGGGCGGTGAG	pMK149	pLB015
	P-LB017	GTCCTCGCCGTAGCCGtACTTCtCCACTCCGAGGAAGACGC		
22	P-LB051	GCGCGAGGGCGGTGAG	pMK149	pLB006
	P-LB007	GTCCTCGCCGTAGCCGtACTTCtgCACTCCGAGGAAGACGCC		
25	P-LB051	GCGCGAGGGCGGTGAG	pMK149	pLB008
	P-LB033	GTGTCCTCGCCGTAGCCctgCTTCGtCACTCCGAGGAAGAC		
27	P-LB032	GTCTTCCTCGGAGTGaCGAAGcagGGCTACGGCGAGGACAC	pMK149	pLB008
	P-LB011	GACGCCTTCGGAGAAGCCtAACCCGTCGGCGCCGG		
30	P-LB016	GCGTCTTCCTCGGAGTGGaGAAGTaCGGCTACGGCGAGGAC	pMK149	pLB015
	P-LB015	GGGTGACGCCTTCGGAGtAGCCtAACCCGTCGGCGCCGG		
32	P-LB034	GGGCCAGCAACGGGCTTcCCGCGCCGAGCGGG	pMK149	pLB008
	P-LB050	TCCGCTCGCCGCGCC		
35	P-LB020	CCGGCGCCGACGGGTaCGGCTggTCCGAAGGCGTCACCCTG	pMK149	pLB005
	P-LB050	TCCGCTCGCCGCGCC		
36	P-LB022	CCGGCGCCGACGGGTTaGGCaTgTCCGAAGGCGTCACCCTG	pMK149	pLB006
	P-LB050	TCCGCTCGCCGCGCC		
37	P-LB014	CCGGCGCCGACGGGTTaGGCTaCTCCGAAGGCGTCACCC	pMK149	pLB015
	P-LB050	TCCGCTCGCCGCGCC		

Table S9. gBlocks used for In-Fusion cloning

gblock	Sequence 5'-3'
pLB007_ gblock	GGCGTATTCCTCGGAGTGCAGAAAAAGGGATATGGCGAAGACACGGCTGCAGCTGAGGACGTCGAGGGATACAGTGTTACCGGCGTGGCGCCTGCCGTGGCTAGTGGT CGAATATCGTACACGATGGGTCTGGAGGGTCCAAGTATTTCCGTAGATACGGCCTGTAGTTCGTCCCTGGTAGCACTGCATTTGGCGGTAGAGTCGCTGCGAAAAGGT GAAAGTTCCATGGCCGTGGTAGGTGGTGCTGCGGTAATGGCAACACCTGGAGTATTCGTGGACTTTTCCCGTCAACGTGCGTTGGCAGCCGACGGTCGTAGTAAGGCG TTTGGCGCGGGAGCGGATGGTTGGGGATTTAGTGAAGGCGTGACCCTGGTGCTCCTCGAGCGACTGAGCGAAGCTCGACGCAATGGTCATGAGGTTTTGGCAGTGGTC CGCGGTTCGGCGCTCAATCAAGACGGAGCAAGCAATGGTTTGACTGCTCCTAGCGGCCCTGCACAAAGGCGCGTGATCCGGCAGGCGCTGGAGTCCTGCGGTCTGGAG CCGGGCGACGTTGATGCTGTCGAAGCTCACGGTACGGGTACTGCGTTGGGTGATCCGATTGAGGCGAATGCGCTCCTCGATACCTATGGACGAGATCGTGATGCCGAC CGACCGTTGTGGCTGGGATCGGTTAAGTCCAACATTGGACATACTCAAGCTGCGGCCGGTGTTACTGGTTTGCTGAAGGTAGTACTCGCACTCCGTAATGGCGAACTG CCAGCAACCCTCCATGTAGAGGAGCCGACACCACATGTAGATTGGTCGTCCGGAGGTGTTGCGCTCCTGGCCGGAAATCAACCATGGAGGAGGGGAGAGCGAACGCGA CGGGCGAGGGTATCGAGCTTCGGTATTAGCGGG
pLB009_ gblock	GGCGTATTCTTGGGGGTGCAGAAGAACGGCTATGGCGAAGATACTGCGGCCGCAGAAGATGTCGAGGGTTACTCAGTTACCGGAGTCGCGCCCGCCGTAGCGTCTGGG CGCATCTCTTATACCATGGGCCTTGAGGGACCCTCAATCAGTGTTGACACCGCTTGTTCATCAAGCCTGGTCGCGTTGCATTTAGCCGTCGAATCATTACGTAAGGGG GAGTCCAGCATGGCAGTTGTGGGGGGAGCAGCGGTGATGGCCACGCCGGGTGTTTTTGTGGATTTTTCGCGCCAACGTGCACTTGCCGCCGATGGGCGTTCAAAGGCG TTCGGTGCAGGCGCGGATGGGTTAGGATTTAGTGAGGGTGTGACCCTTGTTCTTTTGGAGCGTTTGAGTGAGGCTCGCCGTAACGGACATGAGGTGTTGGCGGTTGTA CGTGGTAGCGCGTTAAATCAAGACGGCGCAAGTAACGGTTTACGCGCACCCTCGGGGC
pLB010_ gblock	CTTCCTTGGTGTGGCGAAATATGGCTATGGAGAGGACACCGCTGCGGCAGAAGATGTGGAGGGTTACTCTGTCACAGGAGTGGCACCTGCGGTCGCTTCCGGGCGTAT TTCCTACACAATGGGGTTAGAAGGTCCCAGTATCTCGGTAGACACTGCATGCTCGTCTTCTCTGGTCGCGTTGCATTTGGCTGTGGAGAGTCTTCGCAAAGGGGAGTC AAGTATGGCTGTAGTCGGTGGGGCCACAGTAATGGCAACGCCCGGGGTCTTTGTAGACTTTTCTCGCCAGCGCGCACTTGCCGCGGACGGACGTTCCAAGGCGTTTGG TGCCGGGGCCGACGGGGCTGGGTTTTCTGAAGGGGTAACATTAGTGTTGCTTGAGCGTTTATCAGAAGCCCGTCGTAATGGACATGAAGTACTTGCGGTAGTCCGCGG TAGTGCGCTGAACCAAGATGGAGCCAGCAACGGTCTGACTGCCCCCTCGGGACCCGCTCAGCGCCGTGTGATTCGCCAAGCGCTGGAATCTTGCGGTTTAGAGCCCGG GGATGTCGACGCCGTAGAAGCCCATGGCACTGGAACAGCTTTGGGTGACCCAATCGAGGCTAACGCTCTTCTTGACACTTATGGTCGCGATCGTGACGCCGATCGTCC GCTGTGGTTAGGTTCGGTCAAATCAAACATTGGCCACACGCAGGCTGCAGCAGGCGTAACGGGACTTTTGAAAGTAGTGCTGGCGCTTCGTAATGGGGAGTTACCAGC TACTTTGCACGTTGAGGAACCCACTCCTCATGTAGATTGGTCGTCGGGCGGCGTTGCCTTACTGGCGGGTAACCAACCGTGGCGCCGTGGTGAACGTACTCGTCGTGC GCGTGTTAGTTCGTTCGGGATCTCTGGG
pLB011_ gblock	GGCGTCTTCTTAGGAGTCCAGAAGTATGGCTATGGGGAGGACACCGCTGCTGCGGAGGACGTAGAAGGTTATTCTGTCACAGGTGTAGCGCCTGCCGTGGCGTCAGGC CGCATCTCTTACACAATGGGGTTAGAAGGTCCTTCAATTAGCGTAGACACCGCTTGCTCATCGTCGTTGGTAGCTCTTCACCTTGCAGTAGAGTCTTTACGCAAGGGG GAGTCTTCCATGGCCGTAGTGGGTGGTGCCTGCGTAATGGCAACCCCTGGCGTGTTCGTAGATTTCAGCCGCCAACGTGCATTAGCAGCAGATGGGCGCAGCAAAGCA TTTGGGGCTGGTGCGGATGGATTAGGTTTTTCCGAAGGGGTTACTTTAGTTCTTTTGGAGCGTTTGTCAGAGGCGCGTCGTAATGGTCATGAGGTGTTAGCAGTGGTT CGCGGCTCTGCCCTGAATCAAGACGGGGCAAGTAACGGCTTGCCTGCCCCTTCAGGG
pLB016_ gblock	GTCTTCTTGGGGGCCAGCTATCATGGTTATGGCCAAGATGCAGTGGTACCTGAGGATTCTGAGGGATATCTGCTTACGGGAAACTCGTCTGCGGTTGTAAGTGGGCGT GTTGCTTATGTACTGGGCTTGGAAGGGCCTGCGGTTACGGTAGATACAGCATGTTCCGCATCTTTGGTAGCTTTGCACAGCGCATGCGGATCTCTTCGTGATGGTGAT TGCGGTCTTGCCGTAGCAGGCGGAGTTAGCGTAATGGCCGGTCCAGAAATCTTTACAGAGTTCTCGCGCCAGGGCGGCCTGGCCGTCGATGGGCGTTGTAAGGCCTTT AGCGCAGAGGCGGATGGTTTCGGTTTCGCTGAAGGGGTAGCTGTGGTCTTGTTACAACGCCTTAGTGATGCTCGCCGTGCGGGCCGCCAGGTCTTAGGGGTTGTAGCG GGAAGTGCAATTAATCAAGATGGTGCATCAAACGGACTGTCGGCGCCTTCGGGCGTC
pLB017_ gblock	GTCTTTTTGGGAGCTTGGTATAGTGGTTATGGGCAAGATGCCGTTGTCCCTGAAGACAGCGAAGGATACTTGCTTACAGGGAACAGTTCCGCCGTCGTGTCGGGCCGC GTTGCTTACGTGTTGGGTTTAGAAGGGCCCGCTGTAACGGTAGATACAGCTTGTTCCGCTAGCCTGGTAGCTTTACATTCCGCCTGTGGCTCACTGCGCGATGGTGAT TGTGGATTAGCAGTGGCAGGAGGGGTGGCGGTAATGGCTGGACCCGAGGTTTTTACCGAATTTTCTCGTCAGGGTGGGTTAGCTGTTGACGGTCGCTGTAAGGCGTTT AGCGCCGAGGCCGATGGGTTCGGCTTTGCCGAGGGTGTTGCAGTAGTTCTTCTTCAGCGTTTGTCCGATGCCCGCCGTGCAGGTCGTCAAGTGCTGGGAGTTGTCGCG GGTTCTGCGATTAACCAAGATGGCGCGAGCAACGGACTTCGCGCCCCATCGGGCGTC
pLB018_ gblock	GTCTTTCTGGGGGCATGGTATCATGGGTACGGCCAAGATGCCGTGGTGCCAGAGGACTCGGAGGGCTATTTATTAACAGGTAATTCTTCAGCGGTAGTGAGTGGGCGT GTCGCCTACGTTCTGGGGTTAGAGGGGCCAGCTGTCACGGTGGATACAGCATGCAGTGCTTCGTTGGTCGCTTTACATTCGGCCTGTGGTTCTTTGCGTGATGGTGAT TGCGGTCTGGCAGTAGCAGGAGGGGTAACTGTCATGGCCGGTCCTGAAGTGTTTACTGAATTCTCTCGTCAAGGAGGGTTGGCCGTAGATGGCCGCTGTAAGGCATTT TCCGCTGAAGCAGACGGGTTCGGGTTCGCAGAAGGAGTGGCAGTCGTTTTGCTGCAGCGCTTATCGGATGCTCGTCGCGCTGGACGCCAGGTGTTAGGAGTGGTGGCG

gblock	Sequence 5'-3'
	GGCTCCGCGATCAATCAGGATGGCGCTAGCAACGGCCTTACAGCCCCTTCTGGAGTC
pLB019_ gblock	GTCTTTTTAGGTGCCTCATATCATGGATATGGCCAGGATGCTGTCGTTCCCGAGGATTCCGAGGGCTATCTGCTTACGGGTAACTCTTCTGCGGTAGTAAGCGGACGT GTAGCTTATGTCCTTGGCTTAGAAGGACCTGCTGTGACTGTCGACACAGCGTGCTCCGCCAGCTTAGTTGCGCTTCATAGTGCGTGTGGTAGTTTACGCGACGGAGAC TGTGGTCTGGCCGTCGCGGGAGGAGTTCATGTAATGGCAGGCCCAGAGGTGTTTACTGAGTTTAGCCGTCAAGGAGGTTTAGCGGTAGACGGACGTTGCAAGGCCTTC AGCGCCGAGGCTGACGGGTTCGGATTTGCAGAAGGGGTAGCTGTGGTACTGCTGCAACGCTTGTCAGACGCCCGTCGCGCAGGACGTCAGGTATTGGGAGTAGTTGCT GGGTCTGCTATTAACCAGGATGGCGCGTCAAACGGGTTATCTGCCCCGTCGGGGGTC
pLB021_ gblock	GTCTTTTTAGGCGCGTGGTACTCAGGTTACGGCCAGGACGCTGTTGTGCCCGAAGATTCAGAGGGTTACCTTTTAACCGGGAACTCCAGTGCAGTCGTAAGTGGTCGC GTAGCCTACGTTTTAGGTTTAGAGGGTCCCGCCGTCACTGTGGATACGGCGTGTAGCAGTTCCTTAGTAGCGTTGCACAGTGCTTGCGGATCATTACGCGATGGTGAC TGTGGCCTGGCAGTAGCGGGTGGTGTAGCCGTTATGGCGGGCCCAGAAGTTTTCACCGAATTTTCTCGTCAAGGGGGTTTAGCTGTTGACGGCCGCTGTAAGGCTTTC TCTGCAGAGGCTGATGGTTTTGGCTTTGCAGAGGGGGTAGCCGTAGTCCTGCTGCAGCGTTTATCGGACGCCCGTCGCGCTGGCCGCCAGGTTCTGGGCGTAGTTGCA GGTTCAGCGATCAATCAAGATGGCGCAAGTAACGGCCTTACTGCCCCTTCCGGCGTC
pLB022_ gblock	GTCTTCTTGGGGGCGGCGTACTCTGGATATGGTCAAGATGCGGTGGTTCCCGAGGACTCTGAGGGGTACTTGTTGACTGGCAATAGTAGCGCTGTAGTCAGCGGTCGT GTCGCGTACGTGCTTGGTCTTGAGGGCCCAGCCGTAACTGTCGATACTGCATGTTCAGCCAGCCTTGTGGCCCTGCATTCGGCATGCGGATCCTTACGTGACGGAGAC TGCGGACTTGCGGTGGCTGGTGGGGTTTCGGTAATGGCAGGCCCGGAACTTTTTACGGAGTTTTCGCGCCAAGGTGGCTTAGCAGTAGATGGCCGTTGTAAGGCATTT AGTGCCGAAGCGGATGGCTTTGGAATGGCCGAAGGGGTCGCTGTCGTCCTGCTTCAACGTTTGTCGGACGCTCGTCGTGCGGGTCGCCAGGTACTTGGAGTAGTTGCC GGATCAGCAATCAACCAGGATGGGGCTTCAAACGGGTTAGAAGCACCGTCCGGAGTC
pLB023_ gblock	GTCTTTCTTGGCGCGGGGTATCACGGGTACGGACAGGACGCAGTAGTTCCCGAAGATAGCGAAGGTTACCTGTTGACCGGAAACAGCAGTGCCGTGGTCAGCGGACGT GTCGCGTATGTTCTTGGTTTAGAAGGACCTGCGGTGACTGTTGATACTGCCTGTAGCGCAAGTCTTGTTGCTTTACATAGTGCCTGTGGTTCTTTACGTGATGGAGAC TGTGGGCTGGCGGTGGCCGGAGGCGTGCATGTCATGGCGGGGCCCGAAGTGTTTACAGAGTTCTCACGCCAAGGAGGCCTTGCGGTAGACGGTCGCTGTAAGGCTTTC TCGGCTGAGGCGGATGGTTTCGGCTTTGCGGAAGGGGTCGCCGTTGTATTACTTCAGCGTCTTTCAGATGCTCGTCGCGCCGGTCGTCAGGTCTTGGGCGTGGTAGCG GGATCTGCAATTAATCAGGATGGAGCTAGCAATGGTTTACAGGCTCCGAGCGGCGTC
pLB024_ gblock	GTCTTCCTTGGGGCGTGGTATCAAGGTTACGGTCAGGACGCCGTAGTTCCTGAAGATTCCGAAGGCTATTTGTTAACGGGGAATTCGTCTGCGGTGGTTTCTGGTCGT GTAGCTTACGTCTTAGGGCTTGAGGGACCTGCTGTCACAGTCGATACAGCTTGTAGCGCATCTTTAGTTGCACTGCACTCGGCATGTGGCTCTCTTCGTGATGGAGAC TGCGGATTAGCAGTTGCGGGGGGCGTCACCGTAATGGCCGGCCCTGAAGTCTTCACCGAATTTTCGCGTCAAGGAGGTCTGGCAGTCGACGGACGTTGTAAGGCATTT AGTGCAGAAGCCGATGGTTTTGGGTTCGCCGAAGGTGTCGCCGTTGTGCTGCTGCAGCGTCTGTCAGACGCGCGTCGCGCCGGACGCCAGGTATTGGGTGTGGTAGCG GGAAGTGCGATCAACCAAGATGGCGCGTCTAATGGCTTGAGTGCCCCCAGTGGGGTGGCGCAACAGCGTGTGATCCGTAAAGCCTGGGCACGCGCAGGAATTACTGGA GCGGATGTAGCCGTGGTAGAGGCGCACGGTACAGGGACACGTCTGGGAGACCCAGTCGAAGCGTCCGCTTTGTTAGCGACGTACGGAAAATCACGTGGCTCTAGTGGT CCCGTACTTTTGGGCTCGGTTAAGTCTAACATTGGGCACGCCCAAGCTGCAGCGGGAGTGGCGGGCGTAATTAAGGTTGTCTTAGGGTTAAATCGCGGTTTGGTCCCG CCCATGTTATGTCGCGGTGAACGCAGTCCACTGATTGAATGGAGCTCCGGCGGTGTGGAGCTGGCTGAAGCTGTCAGCCCCTGGCCACCTGCCGCTGACGGAGTGCGT CGTGCTGGAGTAAGCTCTTTTGGGGTTTCGGGG
pLB026_ gblock	GTCTTTTTGGGCGCCGCATACCAAGGTTACGGTCAGGATGCCGTTGTTCCCGAAGACTCCGAGGGTTACCTGCTTACTGGTAATAGCAGTGCGGTGGTCTCAGGTCGC GTAGCATACGTGTTGGGTCTGGAGGGGCCTGCCGTCACAGTCGACACGGCTTGCAGTGCGAGTTTAGTTGCTTTACACAGTGCTTGTGGCAGTCTTCGTGATGGTGAT TGCGGATTAGCCGTTGCTGGTGGTGTGTCTGTGATGGCTGGGCCGGAGCTTTTTACTGAGTTTAGCCGTCAAGGGGGCCTGGCAGTAGATGGACGTTGCAAGGCGTTT TCCGCCGAGGCAGACGGGTTCGGAATGGCTGAGGGGGTGGCGGTGGTCCTTCTTCAACGCCTTTCAGATGCGCGTCGTGCAGGGCGTCAAGTTTTAGGGGTAGTCGCC GGTTCTGCAATTAACCAGGATGGGGCCAGCAACGGGTTAGCGGCACCTTCTGGGGTC
pLB027_ gblock	GTCTTTCTTGGGGCGACTTACCAGGGGTACGGTCAAGACGCGGTTGTGCCCGAGGACTCGGAAGGTTACTTGTTAACCGGAAATAGTTCAGCGGTCGTGAGTGGGCGC GTGGCCTATGTCTTGGGCTTGGAAGGCCCGGCAGTGACCGTGGACACAGCTTGCAGCGCAAGTTTAGTCGCTCTGCATAGTGCCTGTGGATCTTTACGCGATGGCGAT TGCGGCTTGGCTGTCGCCGGTGGCGTCCATGTTATGGCTGGCCCAGAGATGTTTACTGAGTTCTCTCGCCAGGGAGGCTTGGCAGTTGATGGGCGCTGCAAAGCATTC TCTGCGGAGGCGGACGGTTTTGGATTCGCGGAGGGGGTAGCTGTAGTATTGTTACAACGTTTGAGCGATGCCCGTCGTGCTGGCCGTCAGGTTCTTGGTGTAGTGGCC GGTTCAGCTATCAACCAAGACGGTGCATCCAATGGTTTGTCCGCCCCAAGTGGCGTC

Table S10. Amino acid sequences of newly generated substrate-donating modules

Construct	Amino acid sequence
MK178 (5)KS1-AT1-KR2-ACP2-SZ3	MSGDNGMTEEKLRRYLKRTVTELDSVTARLREVEHRAGEPVAVVAMACRLPGGVSTPEEFWELLSEGRDAVAGLPTDRGWDLDSLFH PDPTRSGTAHQRGGGFLTEATAFDPAFFGMSPREALAVDPQQRLMLELSWEVLERAGIPPTSLQASPTGVFVGLIPQEYGPRLAEGG EGVEGYLMTGTTTSVASGRIAYTLGLEGPAISVDTACSSSLVAVHLACQSLRRGESSLAMAGGVTVMPTPGMLVDFSRMNSLAPDGR CKAFSAGANGFGMAEGAGMLLLERLSDARRNGHPVLAVLRGTAVNSDGASNGLSAPNGRAQVRVIQQALAESGLGPADIDAVEAHGT GTRLGDPIEARALFEAYGRDREQPLHLGSVKSNLGHTQAAAGVAGVIKMVLAMRAGTLPRTLHASERSKEIDWSSGAISLLDEPEPW PAGARPRRAGVSSFGISGTNAHAIIEEAPQVVEGERVEAGDVVAPWVLSASSAEGLRAQAARLAAHLREHPGQDPRDIAYSLATGRA ALPHRAAFAPVDESAALRVLDGLATGNADGAAVGTSRAQQRAVFVFPGQGWQWAGMAVDLLDTSPVFAAALRECADALEPHLDFEVI PFLRAEAARREQDAALSTERVDVVQPVMFAVMVSLASMWRAHGVEPAAVIGHSQGEIAAACVAGALSLDDAARVVALRSRVIATMPG NKGMASIAAPAGEVRARIGDRVEIAAVNGPRSVVVAGDSDELDRLVASCTTECIRAKRLAVDYASHSSHVETIRDALHAELGEDFHP LPGFVPFFSTVTGRWTQPDELDAGYWYRNLRRTVRFADAVRALAEQGYRTFLEVSAHPILTAAIEEIGDGSGADLSAIHSLRRGDGS LADFGEALSRAFAAGVAVDWESVHLGTGARRVPLPTYPFQRERVWLLPDRTTPRDELDGWFYRVDWTEVPRSEPAALRGRWLVVVPE GHEEDGWTVEVRSALAEAGAEPEVTRGVGGLVGDCAGVVSLLALEGDGAVQTLVLVRELDAEGIDAPLWTVTFGAVDAGSPVARPDQ AKLWGLGQVASLERGPRWTGLVDLPHMPDPELRGRLTAVLAGSEDQVAVRADAVRARRLSPAHVTATSEYAVPGGTILVTGGTAGLG AEVARWLAGRGAEHLALVSRRGPDTEGVGDLTAELTRLGARVSVHACDVSSREPVRELVHGLIEQGDVVRGVVHAAGLPQQVAINDM DEAAFDEVVAAKAGGAVHLDELCSDAELFLLFSSGAGVWGSARQGAYAAGNAFLDAFARHRRGRGLPATSVAWGLWAAGGMTGDEEA VSFLRERGVRAMPVPRALAALDRVLASGETAVVVTDVDWPAFAESYTAARPRPLLDRIVTTAPSERAGEPETESLRDRLAGLPRAER TAELVRLVRTSTATVLGHDDPKAVRATTPFKELGFDSLAAVRLRNLLNAATGLRLPSTLVFDHPNASAVAGFLTSELGGGSGGGSGN EVTTLENDAAFIENENAYLEKEIARLRKEKAALRNRLAHKKLEHHHHHH
MK179 (5)KS1-AT1-KR5-ACP5-SZ3	MSGDNGMTEEKLRRYLKRTVTELDSVTARLREVEHRAGEPVAVVAMACRLPGGVSTPEEFWELLSEGRDAVAGLPTDRGWDLDSLFH PDPTRSGTAHQRGGGFLTEATAFDPAFFGMSPREALAVDPQQRLMLELSWEVLERAGIPPTSLQASPTGVFVGLIPQEYGPRLAEGG EGVEGYLMTGTTTSVASGRIAYTLGLEGPAISVDTACSSSLVAVHLACQSLRRGESSLAMAGGVTVMPTPGMLVDFSRMNSLAPDGR CKAFSAGANGFGMAEGAGMLLLERLSDARRNGHPVLAVLRGTAVNSDGASNGLSAPNGRAQVRVIQQALAESGLGPADIDAVEAHGT GTRLGDPIEARALFEAYGRDREQPLHLGSVKSNLGHTQAAAGVAGVIKMVLAMRAGTLPRTLHASERSKEIDWSSGAISLLDEPEPW PAGARPRRAGVSSFGISGTNAHAIIEEAPQVVEGERVEAGDVVAPWVLSASSAEGLRAQAARLAAHLREHPGQDPRDIAYSLATGRA ALPHRAAFAPVDESAALRVLDGLATGNADGAAVGTSRAQQRAVFVFPGQGWQWAGMAVDLLDTSPVFAAALRECADALEPHLDFEVI PFLRAEAARREQDAALSTERVDVVQPVMFAVMVSLASMWRAHGVEPAAVIGHSQGEIAAACVAGALSLDDAARVVALRSRVIATMPG NKGMASIAAPAGEVRARIGDRVEIAAVNGPRSVVVAGDSDELDRLVASCTTECIRAKRLAVDYASHSSHVETIRDALHAELGEDFHP LPGFVPFFSTVTGRWTQPDELDAGYWYRNLRRTVRFADAVRALAEQGYRTFLEVSAHPILTAAIEEIGDGSGADLSAIHSLRRGDGS LADFGEALSRAFAAGVAVDWESVHLGTGARRVPLPTYPFQRERVWLPIPTGGRARDEDDDWRYQVVWREAEWESASLAGRVLLVTGP GVPSELSDAIRSGLEQSGATVLTCDVESRSTIGTALEAADTDALSTVVSLLSRDGEAVDPSLDALALVQALGAAGVEAPLWVLTRNA VQVADGELVDPAQAMVGGLGRVVGIEQPGRWGGLVDLVDADAASIRSLAAVLADPRGEEQVAIRADGIKVARLVPAPARAARTRWSP RGTVLVTGGTGGIGAHVARWLARSGAEHLVLLGRRGADAPGASELREELTALGTGVTIAACDVADRARLEAVLAAERAEGRTVSAVM HAAGVSTSTPLDDLTEAEFTEIADVKVRGTVNLDELCPDLDAFVLFSSNAGVWGSPGLASYAAANAFLDGFARRRRSEGAPVTSIAW GLWAGQNMAGDEGGEYLRSQGLRAMDPDRAVEELHITLDHGQTSVSVVDMDRRRFVELFTAARHRPLFDEIAGARAEARQSEEGPAL AQRLAALSTAERREHLAHLIRAEVAAVLGHGDDAAIDRDRAFRDLGFDSMTAVDLRNRLAAVTGVREAATVVFDHPTITRLADHYLE RLVGGSGGGSGNEVTTLENDAAFIENENAYLEKEIARLRKEKAALRNRLAHKKLEHHHHHH
MK183 (5)KS1-AT1-KR2-ACP1-SZ3	MSGDNGMTEEKLRRYLKRTVTELDSVTARLREVEHRAGEPVAVVAMACRLPGGVSTPEEFWELLSEGRDAVAGLPTDRGWDLDSLFH PDPTRSGTAHQRGGGFLTEATAFDPAFFGMSPREALAVDPQQRLMLELSWEVLERAGIPPTSLQASPTGVFVGLIPQEYGPRLAEGG EGVEGYLMTGTTTSVASGRIAYTLGLEGPAISVDTACSSSLVAVHLACQSLRRGESSLAMAGGVTVMPTPGMLVDFSRMNSLAPDGR CKAFSAGANGFGMAEGAGMLLLERLSDARRNGHPVLAVLRGTAVNSDGASNGLSAPNGRAQVRVIQQALAESGLGPADIDAVEAHGT GTRLGDPIEARALFEAYGRDREQPLHLGSVKSNLGHTQAAAGVAGVIKMVLAMRAGTLPRTLHASERSKEIDWSSGAISLLDEPEPW PAGARPRRAGVSSFGISGTNAHAIIEEAPQVVEGERVEAGDVVAPWVLSASSAEGLRAQAARLAAHLREHPGQDPRDIAYSLATGRA ALPHRAAFAPVDESAALRVLDGLATGNADGAAVGTSRAQQRAVFVFPGQGWQWAGMAVDLLDTSPVFAAALRECADALEPHLDFEVI PFLRAEAARREQDAALSTERVDVVQPVMFAVMVSLASMWRAHGVEPAAVIGHSQGEIAAACVAGALSLDDAARVVALRSRVIATMPG NKGMASIAAPAGEVRARIGDRVEIAAVNGPRSVVVAGDSDELDRLVASCTTECIRAKRLAVDYASHSSHVETIRDALHAELGEDFHP LPGFVPFFSTVTGRWTQPDELDAGYWYRNLRRTVRFADAVRALAEQGYRTFLEVSAHPILTAAIEEIGDGSGADLSAIHSLRRGDGS LADFGEALSRAFAAGVAVDWESVHLGTGARRVPLPTYPFQRERVWLLPDRTTPRDELDGWFYRVDWTEVPRSEPAALRGRWLVVVPE GHEEDGWTVEVRSALAEAGAEPEVTRGVGGLVGDCAGVVSLLALEGDGAVQTLVLVRELDAEGIDAPLWTVTFGAVDAGSPVARPDQ AKLWGLGQVASLERGPRWTGLVDLPHMPDPELRGRLTAVLAGSEDQVAVRADAVRARRLSPAHVTATSEYAVPGGTILVTGGTAGLG AEVARWLAGRGAEHLALVSRRGPDTEGVGDLTAELTRLGARVSVHACDVSSREPVRELVHGLIEQGDVVRGVVHAAGLPQQVAINDM DEAAFDEVVAAKAGGAVHLDELCSDAELFLLFSSGAGVWGSARQGAYAAGNAFLDAFARHRRGRGLPATSVAWGLWAAGGMTGDEEA VSFLRERGVRAMPVPRALAALDRVLASGETAVVVTDVDWPAFAESYTAARPRPLLDRIVTTAPSERAGEPETESLRDRLASLPAPER EKALFELVRSHAAAVLGHASAERVPADQAFAELGVDSLSALELRNRLGAATGVRLPTTTVFDHPDVRTLAAHLAAELGGGSGGGSGN EVTTLENDAAFIENENAYLEKEIARLRKEKAALRNRLAHKKLEHHHHHH

Construct	Amino acid sequence
MK184 (5)KS1-AT1-KR1-ACP2-SZ3	MSGDNGMTEEKLRRYLKRTVTELDSVTARLREVEHRAGEPVAVVAMACRLPGGVSTPEEFWELLSEGRDAVAGLPTDRGWDLDSLFH PDPTRSGTAHQRGGGFLTEATAFDPAFFGMSPREALAVDPQQRLMLELSWEVLERAGIPPTSLQASPTGVFVGLIPQEYGPRLAEGG EGVEGYLMTGTTTSVASGRIAYTLGLEGPAISVDTACSSSLVAVHLACQSLRRGESSLAMAGGVTVMPTPGMLVDFSRMNSLAPDGR CKAFSAGANGFGMAEGAGMLLLERLSDARRNGHPVLAVLRGTAVNSDGASNGLSAPNGRAQVRVIQQALAESGLGPADIDAVEAHGT GTRLGDPIEARALFEAYGRDREQPLHLGSVKSNLGHTQAAAGVAGVIKMVLAMRAGTLPRTLHASERSKEIDWSSGAISLLDEPEPW PAGARPRRAGVSSFGISGTNAHAIIEEAPQVVEGERVEAGDVVAPWVLSASSAEGLRAQAARLAAHLREHPGQDPRDIAYSLATGRA ALPHRAAFAPVDESAALRVLDGLATGNADGAAVGTSRAQQRAVFVFPGQGWQWAGMAVDLLDTSPVFAAALRECADALEPHLDFEVI PFLRAEAARREQDAALSTERVDVVQPVMFAVMVSLASMWRAHGVEPAAVIGHSQGEIAAACVAGALSLDDAARVVALRSRVIATMPG NKGMASIAAPAGEVRARIGDRVEIAAVNGPRSVVVAGDSDELDRLVASCTTECIRAKRLAVDYASHSSHVETIRDALHAELGEDFHP LPGFVPFFSTVTGRWTQPDELDAGYWYRNLRRTVRFADAVRALAEQGYRTFLEVSAHPILTAAIEEIGDGSGADLSAIHSLRRGDGS LADFGEALSRAFAAGVAVDWESVHLGTGARRVPLPTYPFQRERVWLEPKPVARRSTEVDEVSALRYRIEWRPTGAGEPARLDGTWLV AKYAGTADETSTAAREALESAGARVRELVVDARCGRDELAERLRSVGEVAGVLSLLAVDEAEPEEAPLALASLADTLSLVQAMVSAE LGCPLWTVTESAVATGPFERVRNAAHGALWGVGRVIALENPAVWGGLVDVPAGSVAELARHLAAVVSGGAGEDQLALRADGVYGRRW VRAAAPATDDEWKPTGTVLVTGGTGGVGGQIARWLARRGAPHLLLVSRSGPDADGAGELVAELEALGARTTVAACDVTDRESVRELL GGIGDDVPLSAVFHAAATLDDGTVDTLTGERIERASRAKVLGARNLHELTRELDLTAFVLFSSFASAFGAPGLGGYAPGNAYLDGLA QQRRSDGLPATAVAWGTWAGSGMAEGPVADRFRRHGVIEMPPETACRALQNALDRAEVCPIVIDVRWDRFLLAYTAQRPTRLFDEID DARRAAPQAAAEPRVGALAGLPRAERTAELVRLVRTSTATVLGHDDPKAVRATTPFKELGFDSLAAVRLRNLLNAATGLRLPSTLVF DHPNASAVAGFLTSELGGGSGGGSGNEVTTLENDAAFIENENAYLEKEIARLRKEKAALRNRLAHKKLEHHHHHH
MK185 (5)KS1-AT1-KR1-ACP5-SZ3	MSGDNGMTEEKLRRYLKRTVTELDSVTARLREVEHRAGEPVAVVAMACRLPGGVSTPEEFWELLSEGRDAVAGLPTDRGWDLDSLFH PDPTRSGTAHQRGGGFLTEATAFDPAFFGMSPREALAVDPQQRLMLELSWEVLERAGIPPTSLQASPTGVFVGLIPQEYGPRLAEGG EGVEGYLMTGTTTSVASGRIAYTLGLEGPAISVDTACSSSLVAVHLACQSLRRGESSLAMAGGVTVMPTPGMLVDFSRMNSLAPDGR CKAFSAGANGFGMAEGAGMLLLERLSDARRNGHPVLAVLRGTAVNSDGASNGLSAPNGRAQVRVIQQALAESGLGPADIDAVEAHGT GTRLGDPIEARALFEAYGRDREQPLHLGSVKSNLGHTQAAAGVAGVIKMVLAMRAGTLPRTLHASERSKEIDWSSGAISLLDEPEPW PAGARPRRAGVSSFGISGTNAHAIIEEAPQVVEGERVEAGDVVAPWVLSASSAEGLRAQAARLAAHLREHPGQDPRDIAYSLATGRA ALPHRAAFAPVDESAALRVLDGLATGNADGAAVGTSRAQQRAVFVFPGQGWQWAGMAVDLLDTSPVFAAALRECADALEPHLDFEVI PFLRAEAARREQDAALSTERVDVVQPVMFAVMVSLASMWRAHGVEPAAVIGHSQGEIAAACVAGALSLDDAARVVALRSRVIATMPG NKGMASIAAPAGEVRARIGDRVEIAAVNGPRSVVVAGDSDELDRLVASCTTECIRAKRLAVDYASHSSHVETIRDALHAELGEDFHP LPGFVPFFSTVTGRWTQPDELDAGYWYRNLRRTVRFADAVRALAEQGYRTFLEVSAHPILTAAIEEIGDGSGADLSAIHSLRRGDGS LADFGEALSRAFAAGVAVDWESVHLGTGARRVPLPTYPFQRERVWLEPKPVARRSTEVDEVSALRYRIEWRPTGAGEPARLDGTWLV AKYAGTADETSTAAREALESAGARVRELVVDARCGRDELAERLRSVGEVAGVLSLLAVDEAEPEEAPLALASLADTLSLVQAMVSAE LGCPLWTVTESAVATGPFERVRNAAHGALWGVGRVIALENPAVWGGLVDVPAGSVAELARHLAAVVSGGAGEDQLALRADGVYGRRW VRAAAPATDDEWKPTGTVLVTGGTGGVGGQIARWLARRGAPHLLLVSRSGPDADGAGELVAELEALGARTTVAACDVTDRESVRELL GGIGDDVPLSAVFHAAATLDDGTVDTLTGERIERASRAKVLGARNLHELTRELDLTAFVLFSSFASAFGAPGLGGYAPGNAYLDGLA QQRRSDGLPATAVAWGTWAGSGMAEGPVADRFRRHGVIEMPPETACRALQNALDRAEVCPIVIDVRWDRFLLAYTAQRPTRLFDEID DARRAAPQAAAEPRVGALAALSTAERREHLAHLIRAEVAAVLGHGDDAAIDRDRAFRDLGFDSMTAVDLRNRLAAVTGVREAATVVF DHPTITRLADHYLERLGGGSGGGSGNEVTTLENDAAFIENENAYLEKEIARLRKEKAALRNRLAHKKLEHHHHHH

References:

(1) Dutta, S.; Whicher, J. R.; Hansen, D. a; Hale, W. a; Chemler, J. a; Congdon, G. R.; Narayan, A. R. H.; Håkansson, K.; Sherman, D. H.; Smith, J. L.; et al. Structure of a Modular Polyketide Synthase. *Nature* **2014**, *510* (7506), 512–517.

(2) Kapur, S.; Chen, A. J.; Cane D. E.; Khosla C.; Molecular Recognition between Ketosynthase and Acyl Carrier Protein Domains of the 6-Deoxyerythronolide B Synthase. *Proc. Natl. Acad. Sci.* **2010**, *107* (51), 22066-22071.

(3) Lowry, B.; Robbins, T.; Weng, C.; Brien, R. V. O.; Cane, D. E.; Khosla, C. In Vitro Reconstitution and Analysis of the 6-Deoxyerythronolide B Synthase. *J. Am. Chem. Soc.* **2013**, *135*, 16809–16812.

(4) Klaus, M.; D'Souza, A. D.; Nivina, A.; Khosla, C.; Grininger, M. Engineering of Chimeric Polyketide Synthases Using SYNZIP Docking Domains. *ACS Chem. Biol.* **2019**, *14* (3), 426–433.