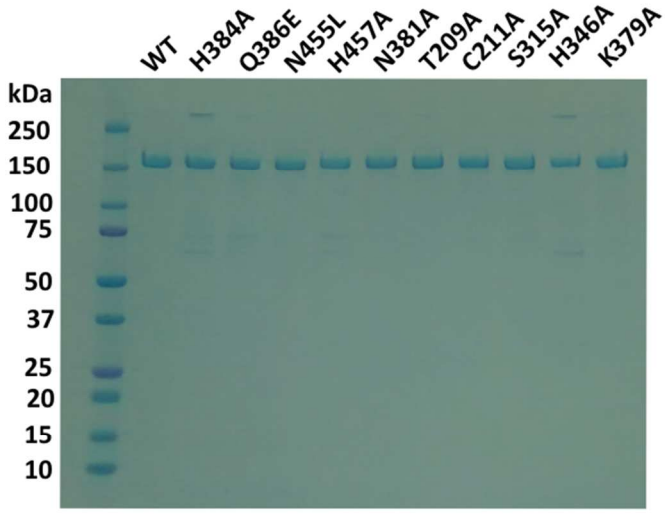
Supporting Information For:

Roles of Conserved Active Site Residues in the Ketosynthase Domain of an Assembly Line Polyketide Synthase

Thomas Robbins[a], Joshuah Kapilivsky[a], David E. Cane[b], Chaitan Khosla*[a]
[a] Departments of Chemistry and Chemical Engineering, Stanford University, Stanford CA 94305, [b] Department of Chemistry, Brown University, Providence RI 02912-9108

*To whom correspondence should be addressed. E-mail: khosla@stanford.edu. Telephone: (650) 723-6538. Fax: (650) 725-7294.



Protein	MW (kDa)	Yield (mg/L of culture)
WT	168.1	2.0
H384A	-	2.1
Q386E	-	0.4
N455L	-	4.6
H457A	-	1.7
N381A	-	3.7
T209A	-	5.1
C211A	-	1.4
S315A	-	1.8
H346A	-	0.5
K379A	-	4.8

Figure S1: Yields and purity of proteins used. SDS-PAGE analysis of individual mutants of DEBS Module 1 used in this study. The yield of each protein from a recombinant *E. coli* culture is summarized in the accompanying Table.

Plasmid	Cloning Method	Primer Name	Primer Sequnce
JK02	Quikchange	JK02-Fwd	CGGTCAAGTCCAACCTCGGCGCCACCCAGGCG
H384A		JK02-Rev	CGCCTGGGTGGCGCCGAGGTTGGACTTGACCG
JK03	Quikchange	JK03-Fwd	GTCCAACCTCGGCCACACCGAGGCGGCCG
Q386E		JK03-Rev	CGGCCGCCTCGGTGTGGCCGAGGTTGGAC
JK04	Quikchange	JK04-Fwd	GGCATCAGCGGCACCCTCGCGCACGCCATCATCGA
N455L		JK04-Rev	TCGATGATGGCGTGCGCGAGGGTGCCGCTGATGCC
JK05	Quikchange	JK05-Fwd	GCATCAGCGGCACCAACGCGGCAGCCATCATCGAGGAAGC
H457A		JK05-Rev	GCTTCCTCGATGATGGCTGCCGCGTTGGTGCCGCTGATGC
JK06	Quikchange	JK06-Fwd	GCTCGGTCAAGTCCGCCCTCGGCCACACCCA
N381A		JK06-Rev	TGGGTGTGGCCGAGGGCGGACTTGACCGAGC
JK07	Quikchange	JK07-Fwd	ATCAGCGTGGACGCCGCGTGCTCGTCCTC
T209A		JK07-Rev	GAGGACGAGCACGCGGCGTCCACGCTGAT
JK08	Quikchange	JK08-Fwd	GTGGACACCGCGGCCTCGTCCTCGCTGGTCG
C211A		JK08-Rev	CGACCAGCGAGGACGAGGCCGCGGTGTCCAC
JK09	Quikchange	JK09-Fwd	CGAGCAACGGGCTGGCGGCGCCCAACG
S315A		JK09-Rev	CGTTGGGCGCCGCCAGCCCGTTGCTCG
JK11	Quikchange	JK11-Fwd	GCCGTCGAGGCGGCCGGCACCGGTACCCGAC
H346A		JK11-Rev	GTCGGGTACCGGTGCCGGCCGCCTCGACGGC
TR11	In-Fusion	TR11-C-term	CTCGGTCGCGTCCAACCTCG
K379A		TR11-N-term	GTTGGACGCGACCGAGCCC
		AmpR-N-term	GCCATACCAAACGACGAGCGTGACAC
		AmpR-C-Term	CTCGTCGTTTGGTATGGCTTCATTC

Table S1 Primers used for the generation of Module 1 Mutants. In all cases, pBL13[1] was used as the PCR template.

Reference

1. Lowry, B., Robbins, T., Weng, C.-H., O'Brien, R. V., Cane, D. E., and Khosla, C. (2013) *JACS 135*, 16809–16812.