Supplementary Information

Bacterial Product Template Domain: An Evolutionary Intermediate between Dehydratase and Aldol Cyclase of Type I Polyketide Synthases

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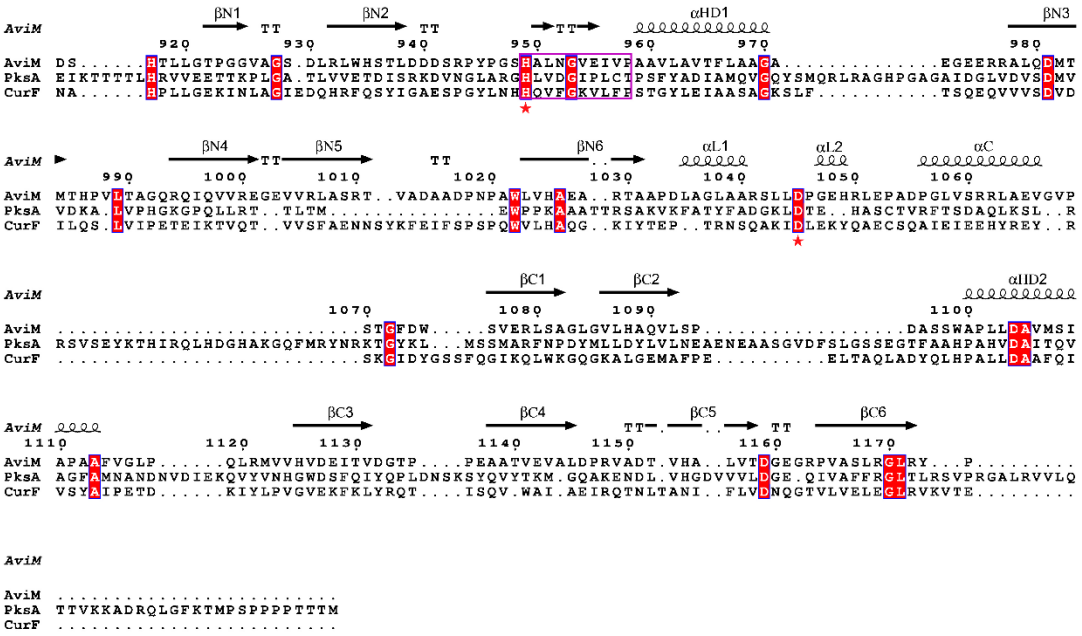

Figure S1. Multiple sequence alignment of AviM PT, PksA PT and CurF. The catalytic dyads are highlighted with red asterisk. The HXXXXXXXXP motif are highlighted with red square.

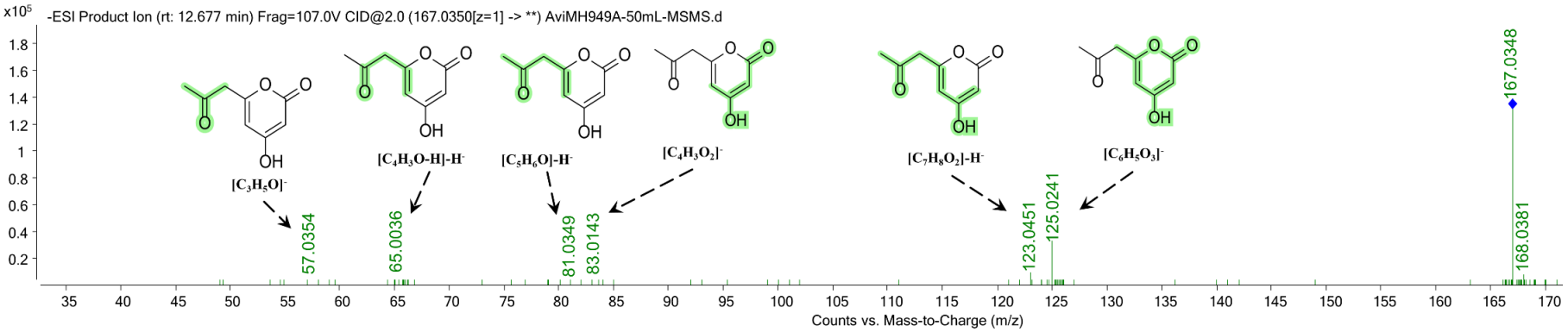

Figure S2. Collision-induced dissociation (CID) mass spectra of precursor ion scan (m/z 167.035, rt: 12.677 min. CE at 2 ev) by LC/ESI-MS. The potential fragments and formulars are labeled in the spectra.

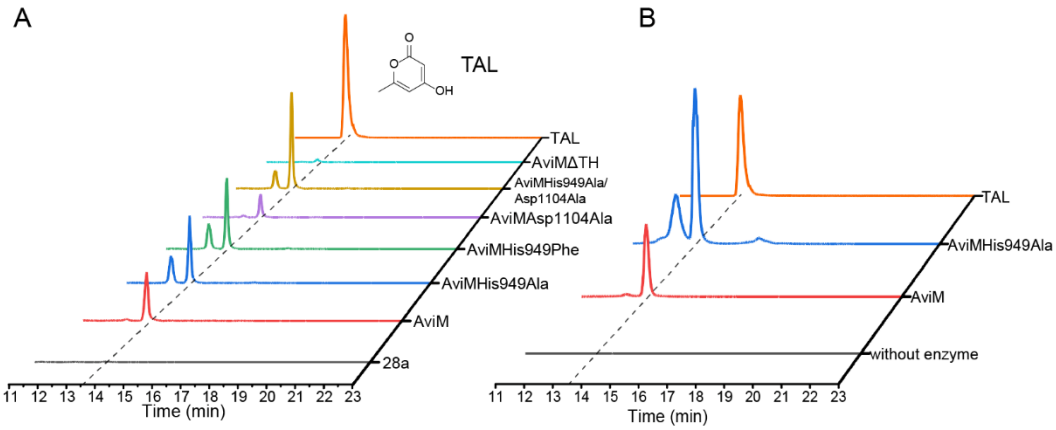

Figure S3. TAL was generated in the *in vivo* (A) and *in vitro* (B) assays. The traces shown are the selected ion monitoring (m/z: 125.0274) in the negative ionization mode of the LC-MS analysis.

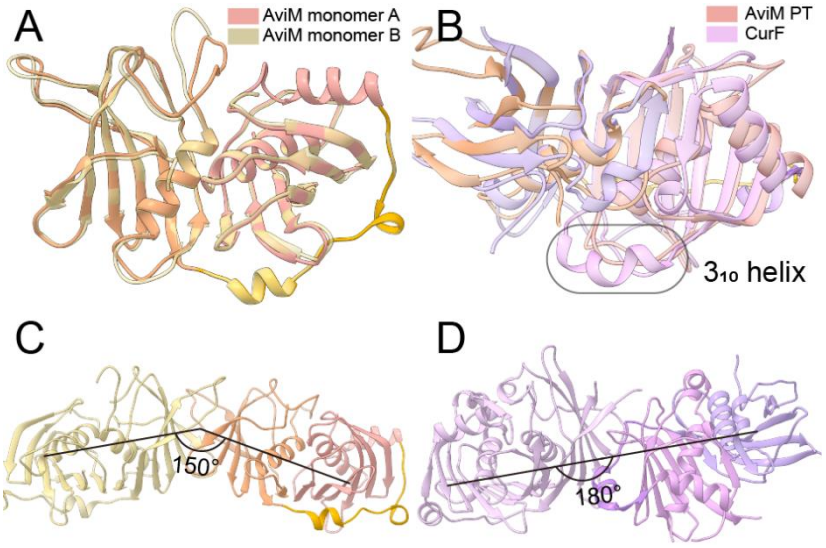

Figure S4. Structure differences between CurF and AviM PT. (A) Two monomers AviM PT were superposed. (B) Comparison of CurF and AviM PT. CurF has an additional 3_{10} helix. (C) The angle formed by two monomers of AviM PT is ~150°. (D) The angle formed by two monomers of CurF is ~180°.

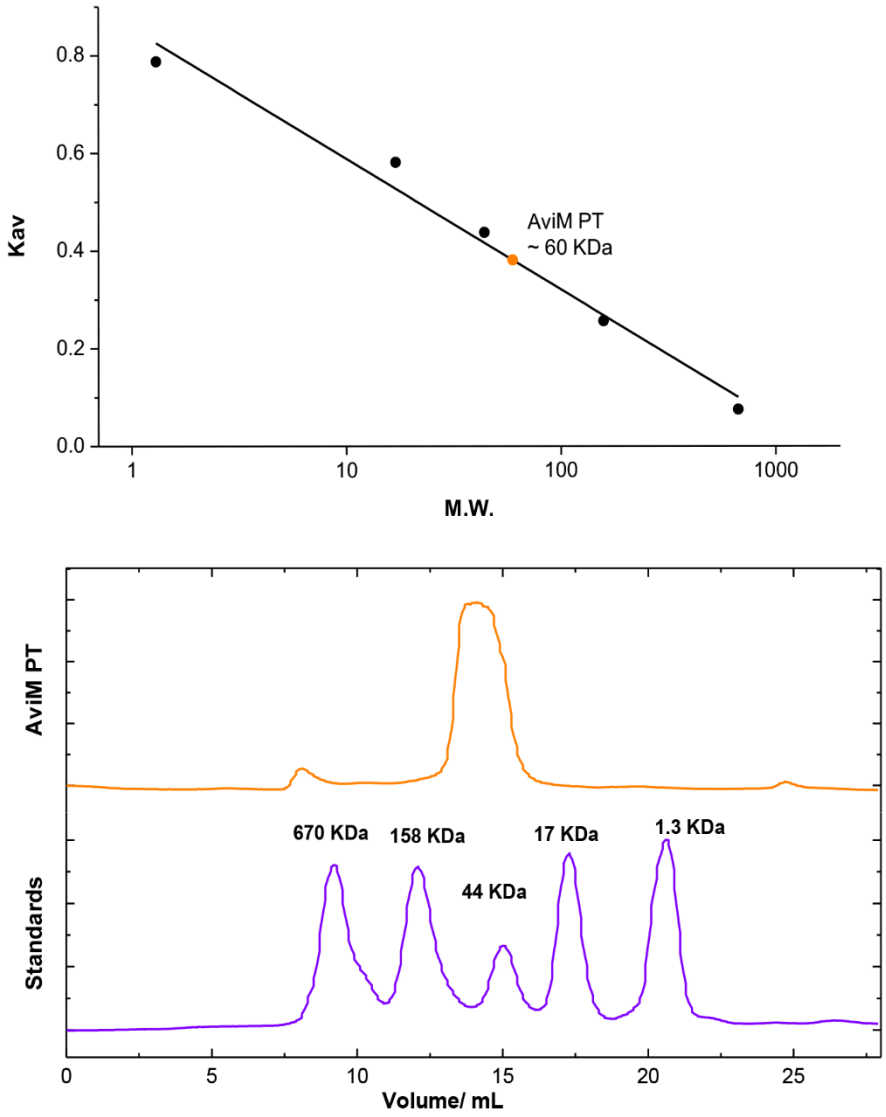

Figure S5. Molecular weight estimate by size-exclusion chromatography. AviM PT migrates at ~60 KDa (expected monomer mass: 29 kDa) compared to standards (FPLC buffer: 10 mM Tris, pH 7.0, 150 mM NaCl).

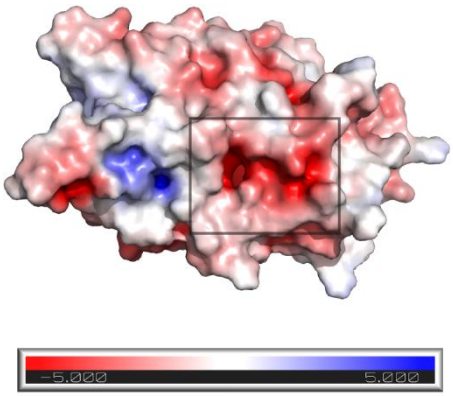

Figure S6. The Electrostatic potential surface of AviM PT. The dark square box indicated the negative substrate tunnel.

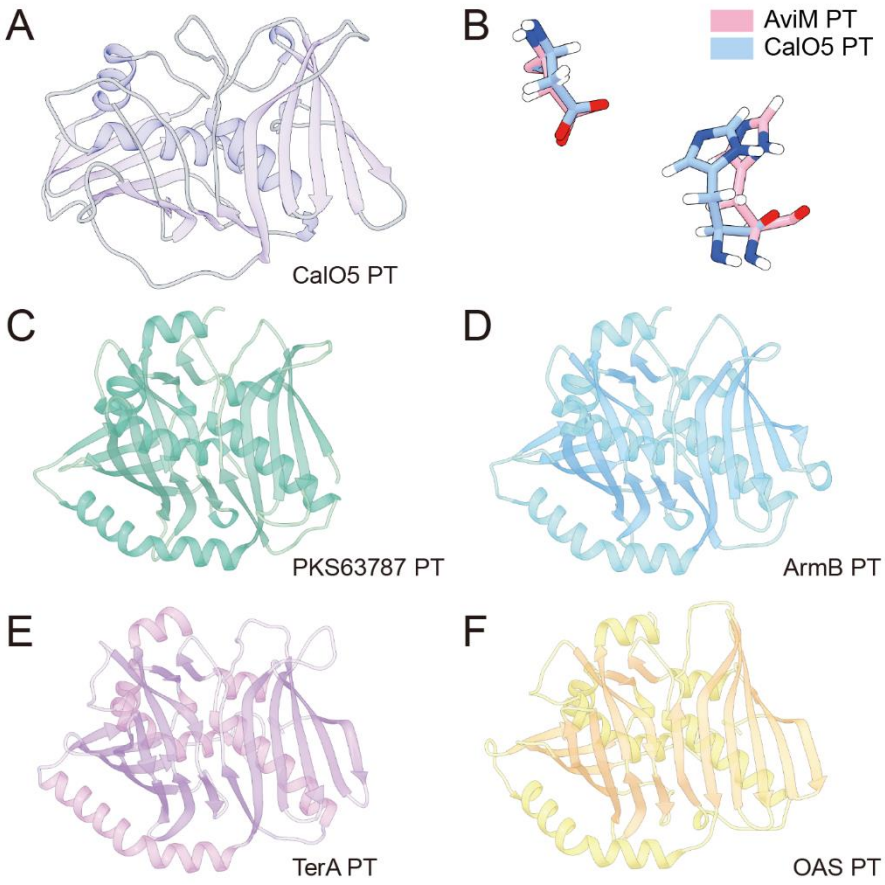

Figure S7. The overall structure of bacterial CalO5 PT and fungal OSAS PTs. All of the structure were constructed by Alphafold2.

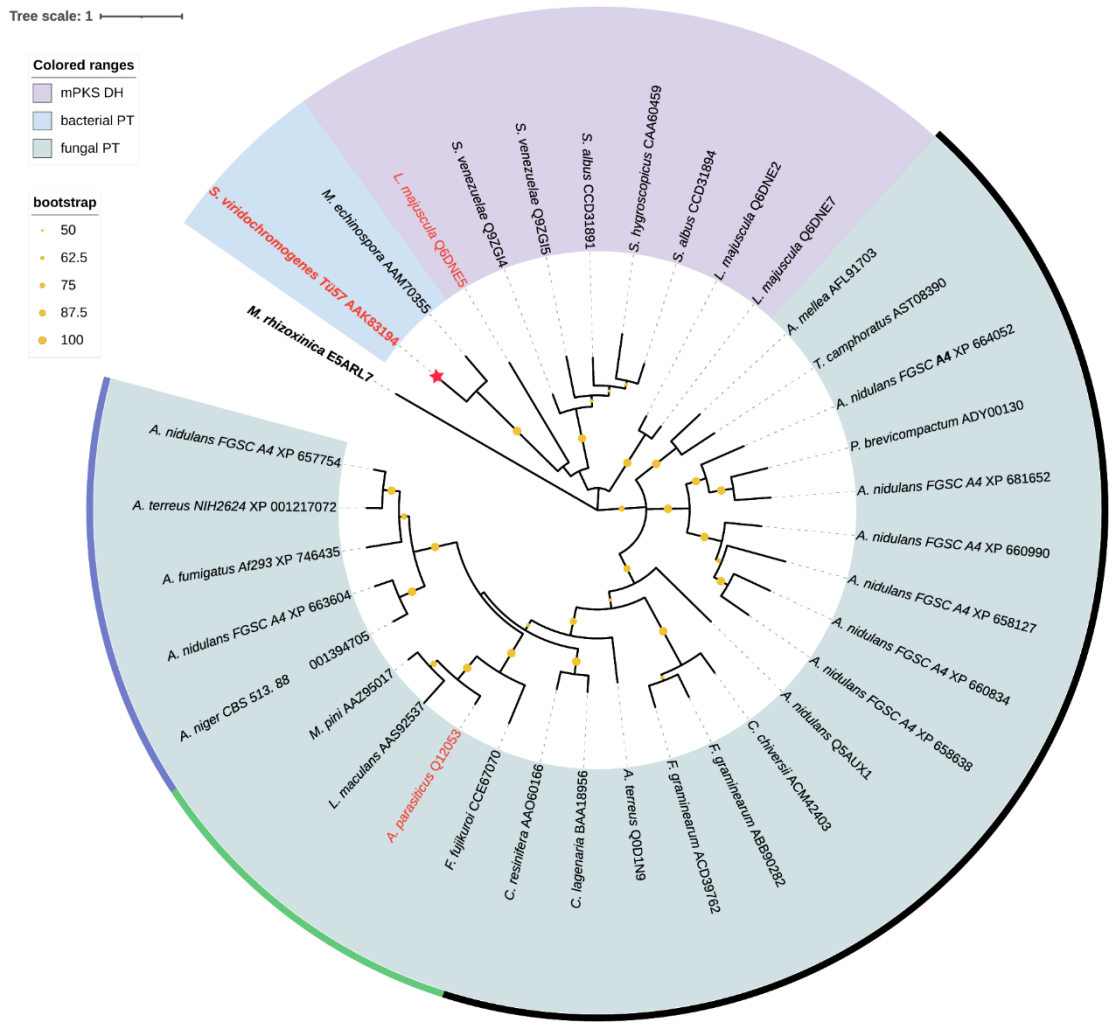

Figure S8. Phylogenetic tree of bacterial PTs, mPKS DHs and fungal PTs. The color range of light blue, light purple and pale green represent the bacterial PTs, modular type I PKS DHs and fungal PTs, respectively. The uncolored range node (*M. rhizoxinica* E5ALR7) is the outgroup branching domain. The yellow dot indicates the clustering with bootstrap value. Sequence of AviM PT is marked by red asterisk and labeled in red. PksA PT (*A. parasiticus* Q12053) and CurF (*L. majuscule* Q6DNE5) are labeled in red. The black, green and dark purple circular represent fungal PTs with C2-C7, C4-C9, C6-C11 cyclization specificity.

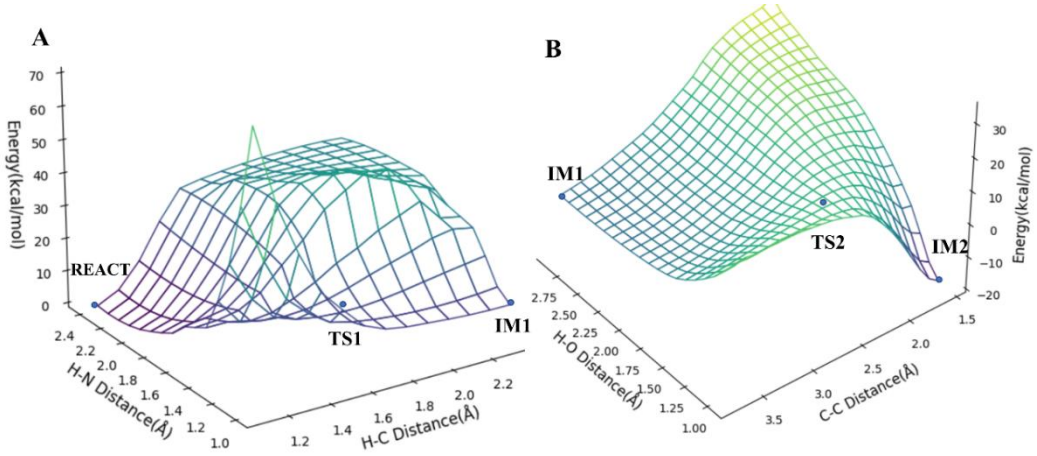

Figure S9 Two-dimensional potential energy surfaces (PESs) in the AviM PT-substrate system: (A)the deprotonation of C2; (B) the subsequent ring cyclization.

Table S1. Primer list

Primer	Sequence (5'→3')
AviMF	gcagatatacatatgaataccggcaacgacgaa
AviMR	gtggtgactagttcaggacgcatggccgtccgc
AviMPTF	gcagatatacatatggcgcgagcggccgagcgc
AviMPTR	tgattcgatgaattcacgggtagcgcaggccgcg
AviMD1104AF	gtcctgggccccgctgctggccgccgtgatgtcgatcgc
AviMD1104AR	ccagcagcggggcccaggacgaggcgtcgggcgagagc
AviMH949FF	cgcccgtacccgggcagcttcgccctcaacggcgtggaga
AviMH949FR	gctgcccgggtacgggcggctgtcgtcgtccagcgtgc
AviMH949AF	cgcccgtacccgggcagcgccgccctcaacggcgtggaga
AviMH949AR	gctgcccgggtacgggcggctgtcgtcgtccagcgtgc
AviMΔTHF	ccgagcgcggccacgacgtcgtggtcgagcagccggcc
AviMΔTHR	gacgtcgtggccgcgctcggccgctcgcgccccggcga

Table S2. Data Collection and Refinement Statistics

Data Collection		
Dataset	Native 316-8	Se-Met Protein
Wavelength (Å)	0.97853	0.97853
Space group	$P2_1$	$P2_1$
Cell dimensions		
a,b,c (Å)	51.679, 58.708, 82.502	51.433, 58.556, 81.741
α, β, γ (º)	90.000, 96.275, 90.000	90.000, 95.907, 90.000
Resolution (Å)	50-2.0(2.00-2.03)	50-2.70 (2.75-2.70)
R_{merge}	0.062(0.445)	0.129 (0.580)
$I/\sigma I$	13.7(2.42)	11.1 (2.0)
Completeness (%)	98.3(97.8)	99.9 (99.6)
Redundancy	3.4(3.5)	3.4 (3.0)
Refinement		
Resolution (Å)	50-2.0	
No. reflections	31071	
R_{work}/R_{free}	0.2022/0.2528	
No. atoms	3744	
B-factors (Å)	49.743	
R.m.s. deviations		
Bond lengths (Å)	0.01	
Bond angles (º)	0.783	