Supporting Information

Morita et al. 10.1073/pnas.1011499107

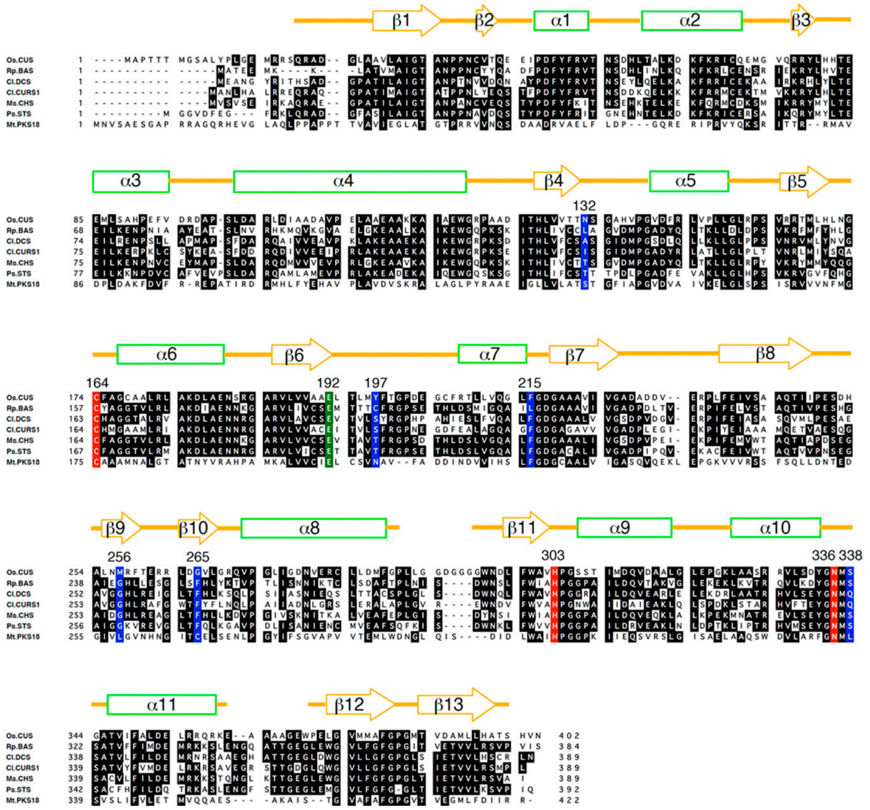

Fig. S1. Comparison of the primary sequence of CUS and other type III PKSs. The secondary structures of CUS are also delineated: α-helices (green rectangles), β-strands (orange arrows), and loops (orange, bold lines) are diagrammed. The catalytic triad Cys-His-Asn residues and the residues thought to be crucial for the functional diversity of type III PKSs are colored in red and blue, respectively (numbering according to *M. sativa* CHS). The Glu residue involved in the electronic hydrogen bond network in CUS and STS and equivalent residues were colored in green. Abbreviations (GeneBank accession numbers): Os.CUS, *Oryza sativa* CUS (AK109558); Rp.BAS, *Rheum palmatum* BAS (AAK82824); Cl.DCS, *C. longa* DCS (AB495006); Cl.CURS1, *C. longa* CURS1 (AB495007); Ms.CHS, *M. sativa* CHS (Os07-g17010.1_ORYZA); Ps.STS, *Pinus sylvestris* STS (AAB24341); Mt.PKS18, *M. tuberculosis* PKS18 (A70958).

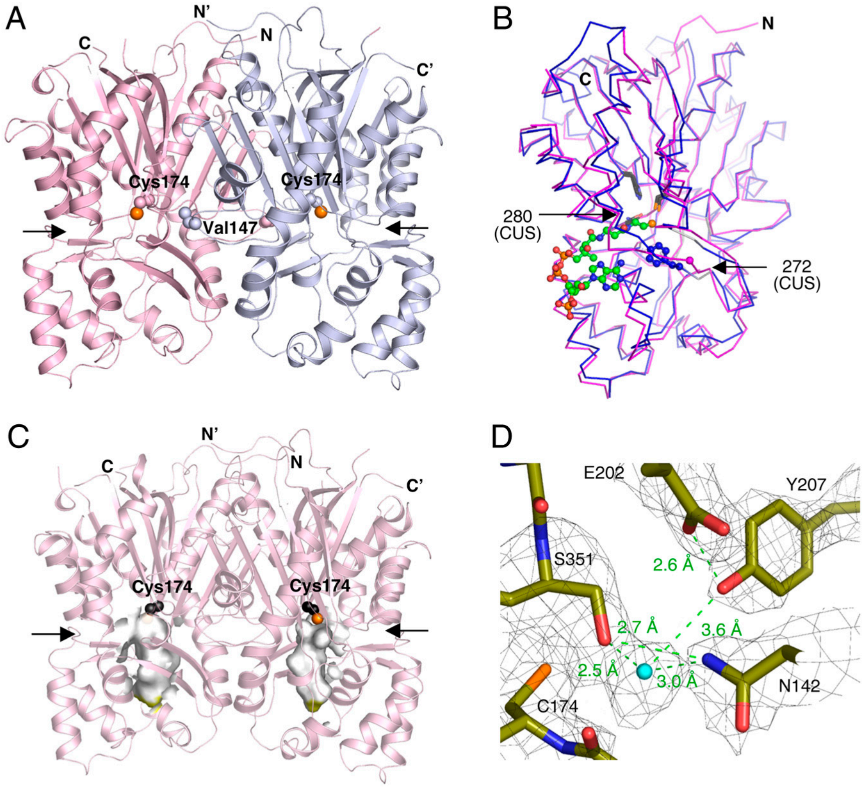

Fig. S2. Overall structure of CUS. (*A*) Both monomer C (purple) and D (blue) were represented by ribbon model. The catalytic Cys174 and Val147 were represented with CPK models, respectively. Arrows indicate the substrate entrance in each monomer. (*B*) Comparison of CUS (purple) and *M. sativa* CHS (blue). The Cys-His-Asn catalytic triad and the CoA-SH bound in *M. sativa* CHS structure were shown by a black stick and ball-and-stick models, respectively. Gly265 of CUS and Phe265 of *M. sativa* CHS are also shown as purple and blue ball-and-stick models, respectively. (*C*) The active sites were indicated by surface model in the overall structure of CUS. The catalytic Cys174 was represented with CPK models. Arrows indicate the substrate entrance in each monomer. (*D*) The F_o-F_c density map of the thioesterase-like hydrogen bond network in monomer C, countered at 2.0 sigma. The water molecule and the hydrogen bonds are indicated with light-blue sphere and green dotted lines, respectively.

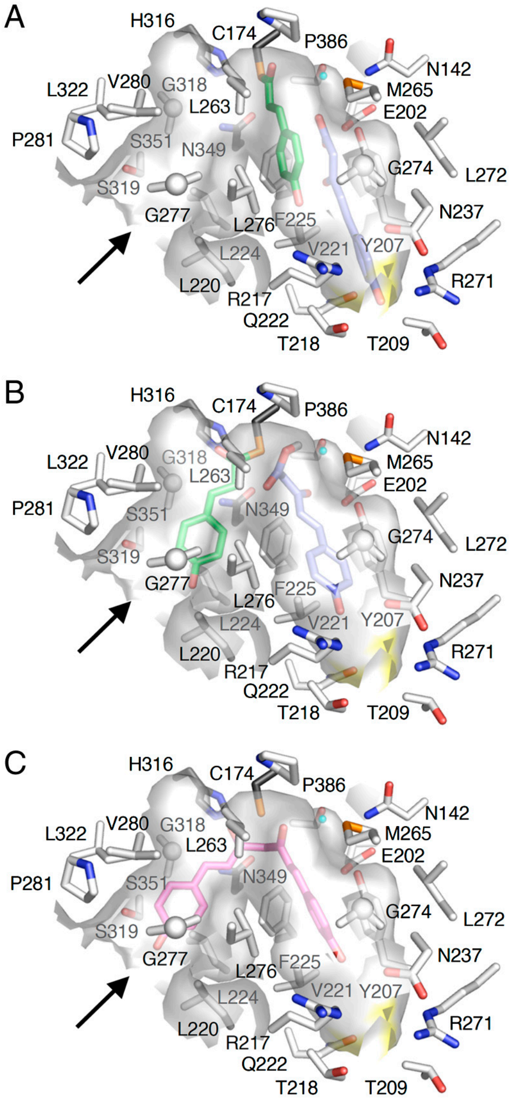

Fig. S3. Three-dimensional models for the binding of the intermediate and product of CUS. (*A*) The coumaroyl monoketide covalently bound to the catalytic center Cys174 (green) and 4-coumaroyldiketide acid (blue) within the active site. (*B*) The coumaroyl monoketide covalently bound to the catalytic center Cys174 (green) within the CoA-binding tunnel and 4-coumaroyldiketide acid (green) within the active site. (*C*) Bisdemethoxycurcumin (purple). The walls of the active-site and a partial CoA-binding tunnel were represented by surface model. The substrate entrances are indicated with arrows. The Cys-His-Asn catalytic triad is shown as a black stick model.

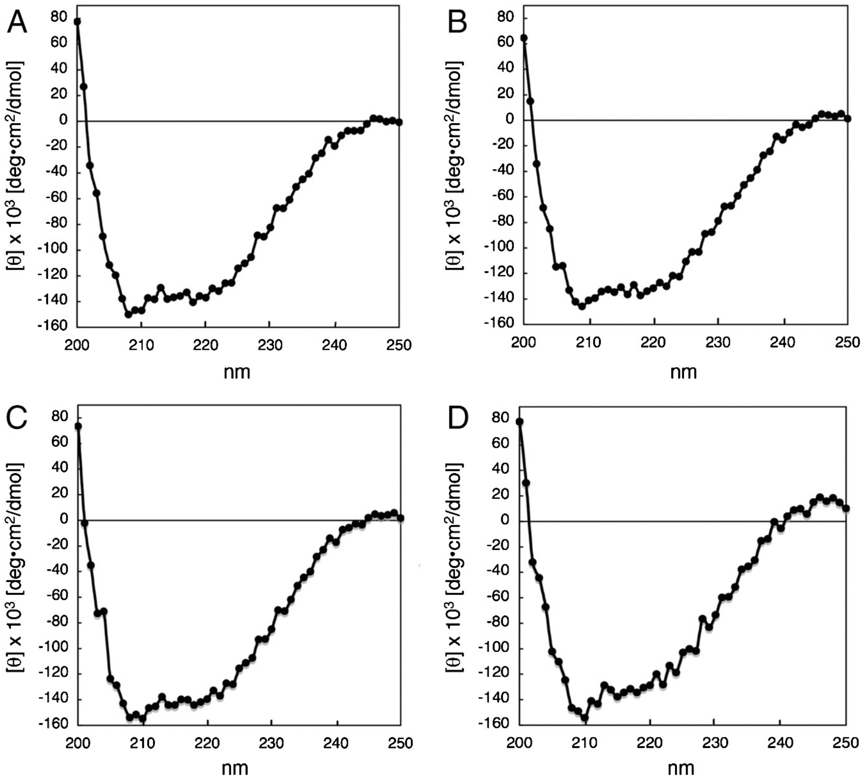

Fig. S4. Circular dichroism (CD) spectra of the wild type and its Y207F, M265L, and G274F mutant enzymes. (*A*) Wild-type CUS. (*B*) The CUS Y207F. (*C*) The CUS M265L mutant. (*D*) The CUS G274F mutant. The CD spectra were recorded at 25 °C with a concentration of 0.22 μM protein in 20 mM HEPES-NaOH (pH 7.0), 100 mM NaCl and 0.2 mM DTT, using a SIMAZU AVIV 202 spectrometer with a 1.0-mm path length cell.

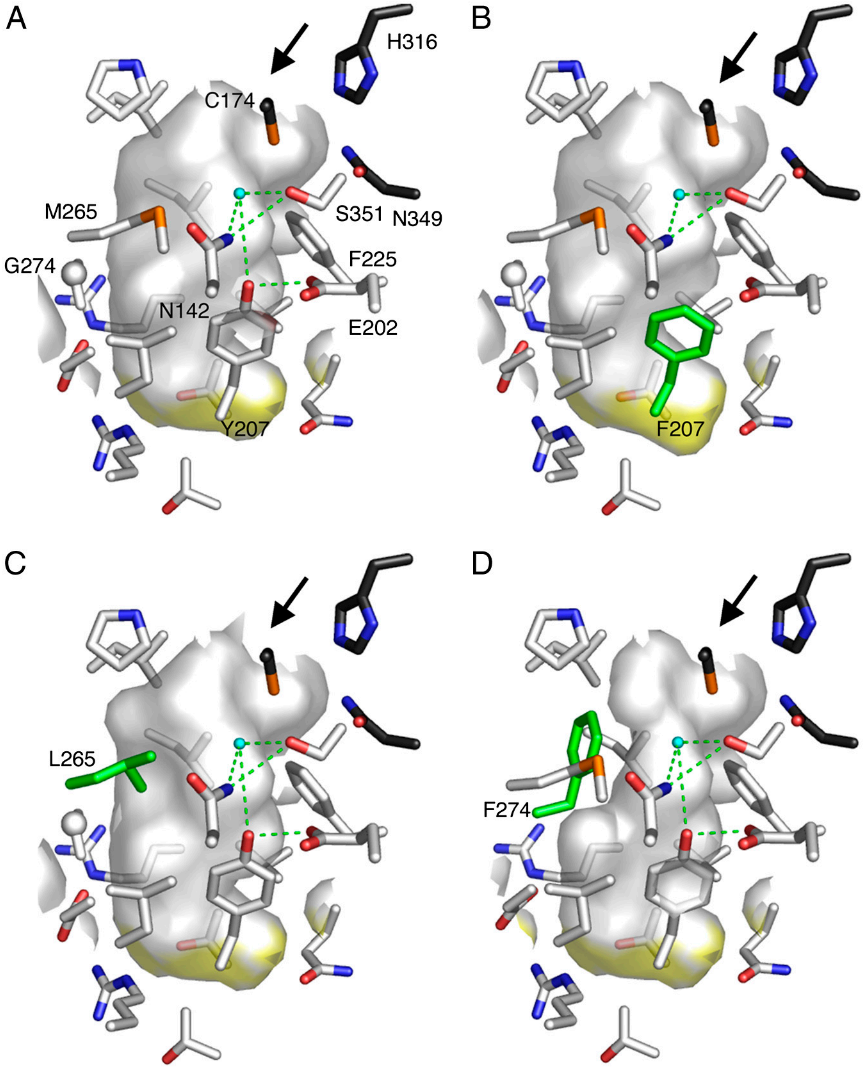

Fig. S5. Three-dimensional homology models of the Y207F, M265L, and G274F CUS mutant enzymes. (*A*) Wild-type CUS. (*B*) The CUS Y207F mutant. (*C*) The CUS M265L mutant. (*D*) The CUS G274F mutant. The active-site architectures were represented by a surface model. The substrate entrances are indicated with arrows. The bottoms of the active site were highlighted as yellow surface. The Cys-His-Asn catalytic triads were shown as a black stick model. The mutated residues were indicated by a green stick model. The water molecule and the hydrogen bond are shown as light-blue sphere and green dotted line, respectively.

Table S1. Data collection and refinement statistics

Data collection	CUS
Space group	$P2_1$
Unit-cell	
a, b, c (Å)	72.7, 97.2, 126.2
α, β, γ (°)	90.0, 103.7, 90.0
Resolution (Å)	50.0–2.5 (2.59–2.50)
Unique reflections	54,813
Redundancy	3.3 (3.2)
Completeness (%)	93.0 (88.6)
$\langle I/(\sigma I)\rangle$	21.7 (5.2)
R_{sym} (%)*	8.9 (32.5)
Refinement	
Resolution (Å)	2.5
$R_{\mathrm{cryst}}/R_{\mathrm{free}}$ (%)†	22.1/27.2
No. atoms	
Protein	11,098
Water	125
Ligand	-
B-factors (Å^2)	
Protein	29.8
Water	20.6
Ligand	-
R.m.s deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.5

Values in parentheses are for the highest resolution shell.

*$R_{\mathrm{sym}} = \mathrm{S}_h\mathrm{S}_i|I(h)_i - \langle I(h)\rangle|/\mathrm{S}_h\mathrm{S}_i I(h)_i$, where $I(h)$ is the intensity of refraction h, S_h is the sum over all reflections, and S_i is the sum over i measurements of reflection h.

†R_{free} was calculated with 5% of data excluded from refinement.