Supplemental information to:

Crystal structure of the first bacterial diterpene cyclase: CotB2

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Table S1 Phasing statistics

Phasing statistics	
resolution [Å] [a]	29.66 - 2.50
	(2.56 - 2.50)
R_{cullis} (acentric) [b]	0.864 (0.964)
phasing power (acentric) [c]	1.042 (0.698)
no. of selenium sites	16

[a] values in parentheses refer to the highest resolution shell.

[b] R_{cullis} = ⟨phase-integrated lack of closure⟩ / $\langle |F_{PH} - F_P| \rangle$

[c] Phasing power = ⟨[$|F_H(calc)|$/phase-integrated lack of closure]⟩

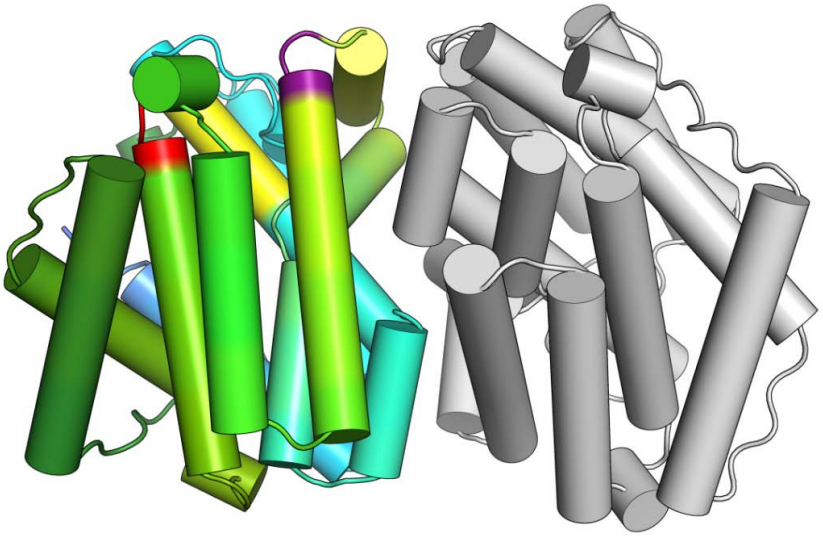

Figure S1 Dimeric arrangement of CotB2. In one monomer the protein is coloured in a gradient from green (N-terminus) to blue (C-terminus). α-helices are drawn as cylinders. The second monomer is drawn in gray. The location of the aspartate-rich motif is indicated in red and the NSE/DTE-motif is marked in orange. The double conformation at the C-terminal end of helix F is indicated in purple.

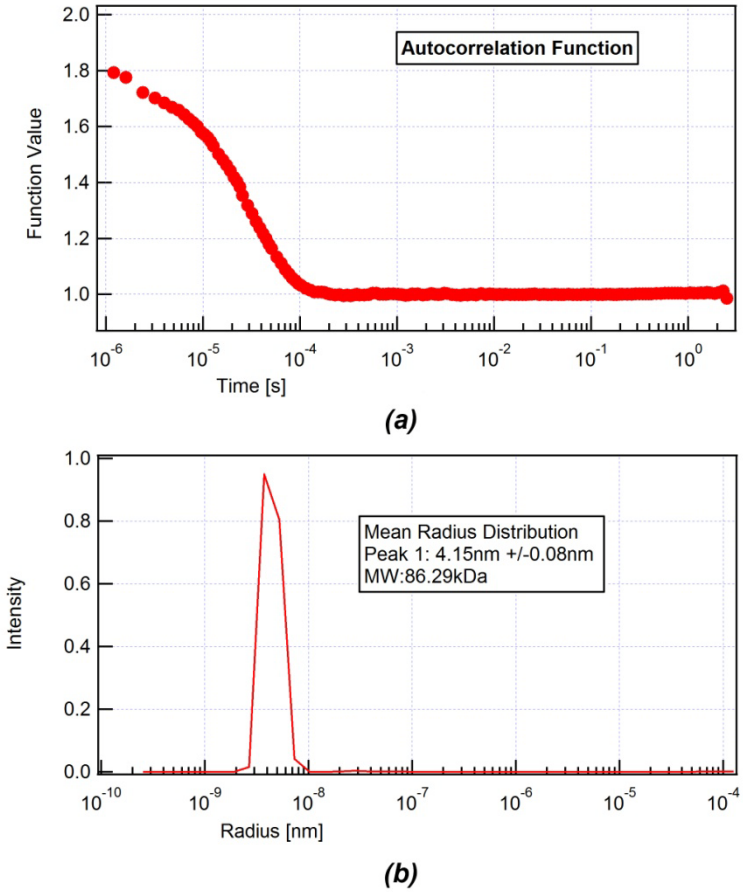

Figure S2 Dynamic light scattering experiments with CotB2. (*a*) The autocorrelation function of experimental data (red circles). The time of the experiments is logarithmically plotted against the amplitude. (*b*) The logarithmic size distribution is plotted versus the intensity. The experimental molecular weight was computed for CotB2 to 86 kDa (theoretical Mw 35.5 kDa per monomer).

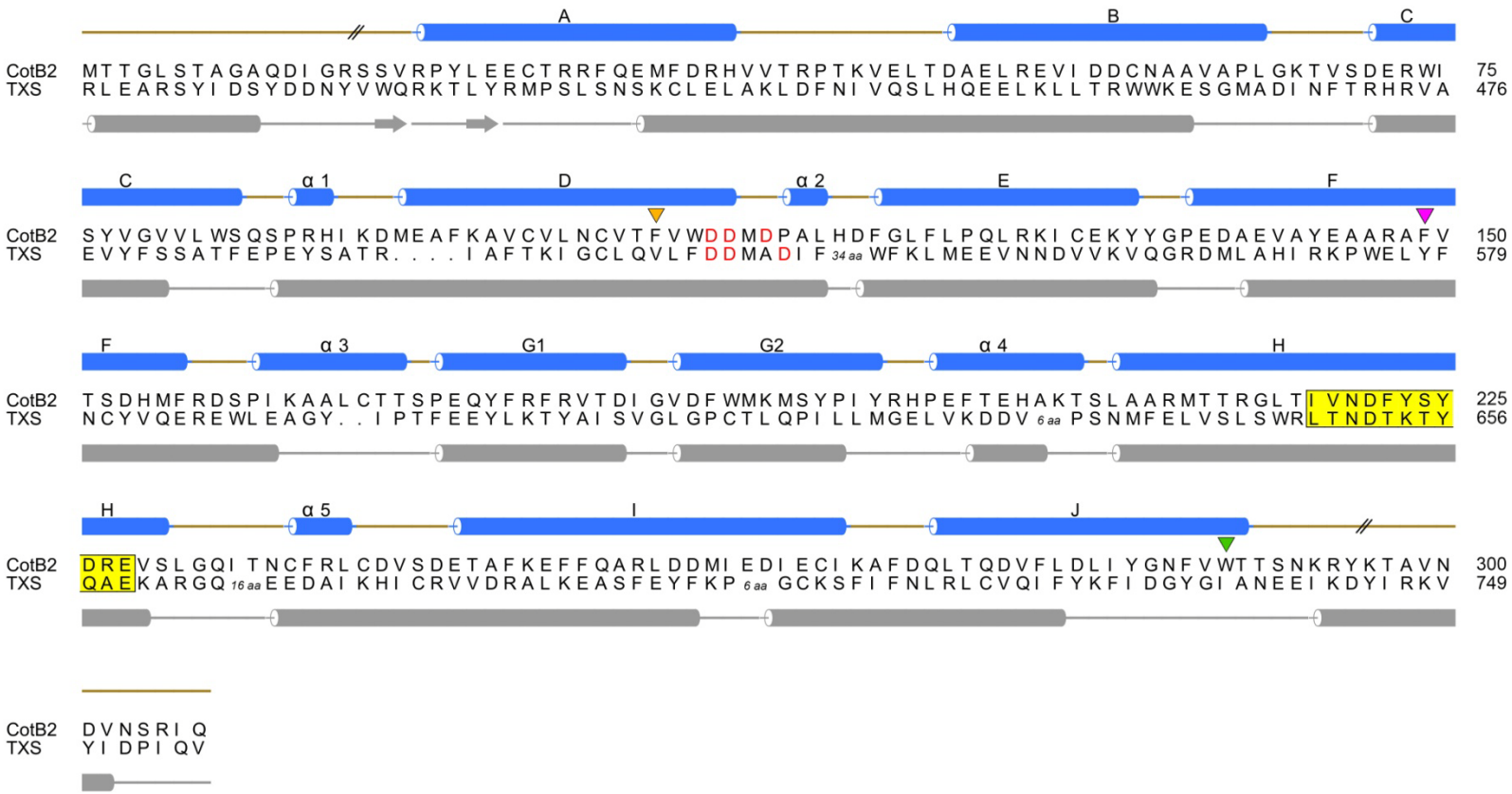

Figure S3 Structure-based alignment of CotB2 and the α-domain of TXS. On top the amino acid sequence of CotB2, its secondary structure elements are depicted. Below the amino acid sequence of TXS as well as the secondary structure elements of TXS. The aspartate-rich motif is highlighted in red and the NSE/DTE-motif in yellow. Residues subjected to mutagenesis are indicated by triangles: F105 (orange), F149 (magenta) and W288 (green).

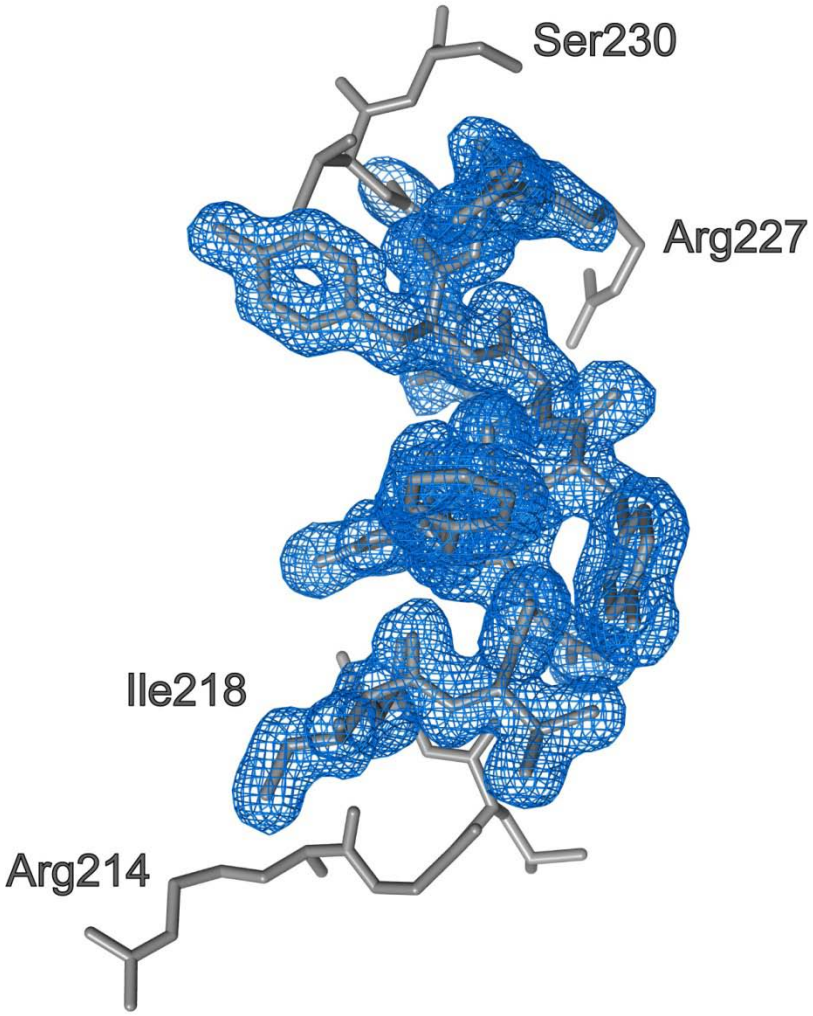

Figure S4 NSE/DTE-motif. The NSE/DTE (^{218}IVNDFYSYDRE^{228}) motif of CotB2 resides on α-helix H. Amino acids are depicted in stick representation. Final m*2Fo*-D*Fc* electron density map is shown for the residues ^{218}IVNDFYSYDRE^{228} contoured at σ = 1.0.

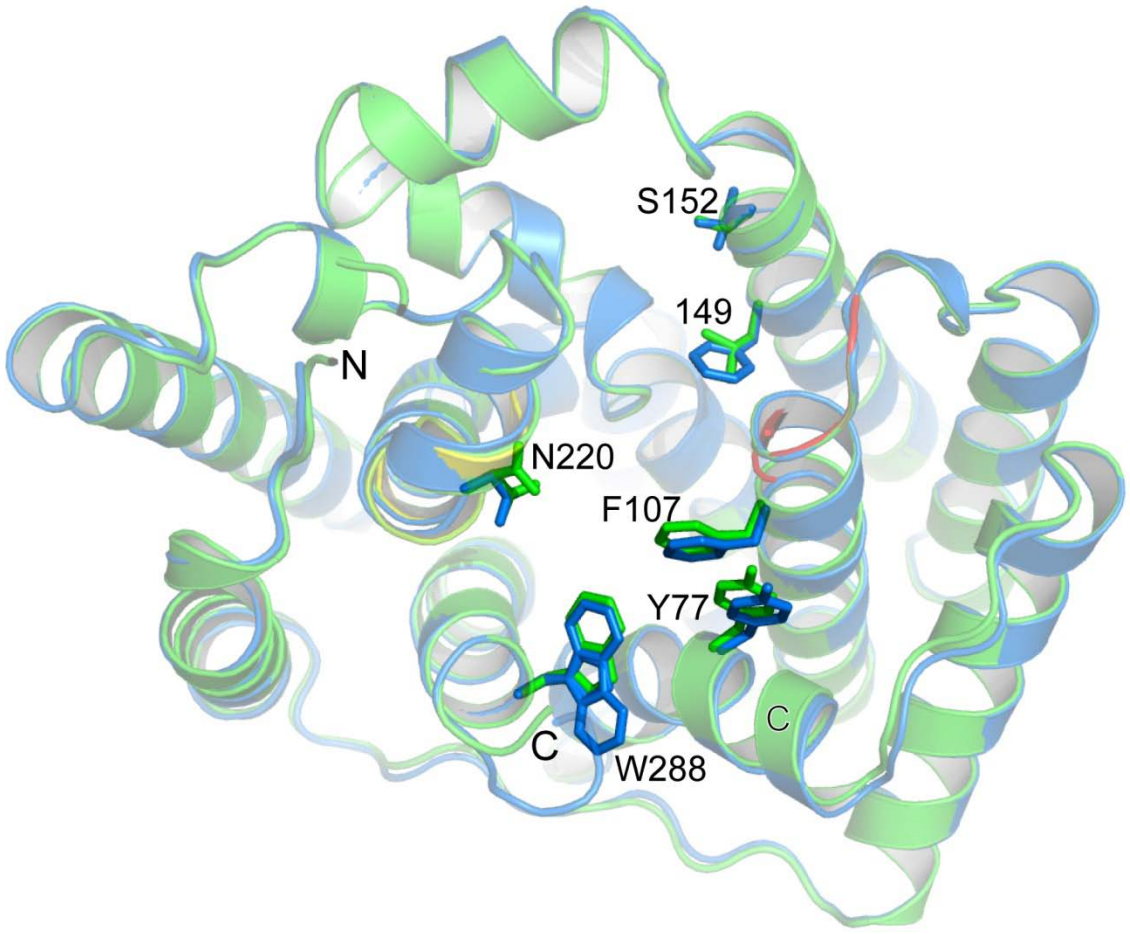

Figure S5 Superposition of CotB2^{wt} and CotB2^{F149L}. View into the active site of CotB2. CotB2^{wt} is drawn in blue and CotB2^{F149L} in green. Active site residues undergoing conformational changes upon mutation of phenylalanine to leucine at position 149 are shown in stick representation. The aspartate-rich motif is highlighted in red and the NSE/DTE-motif in yellow.

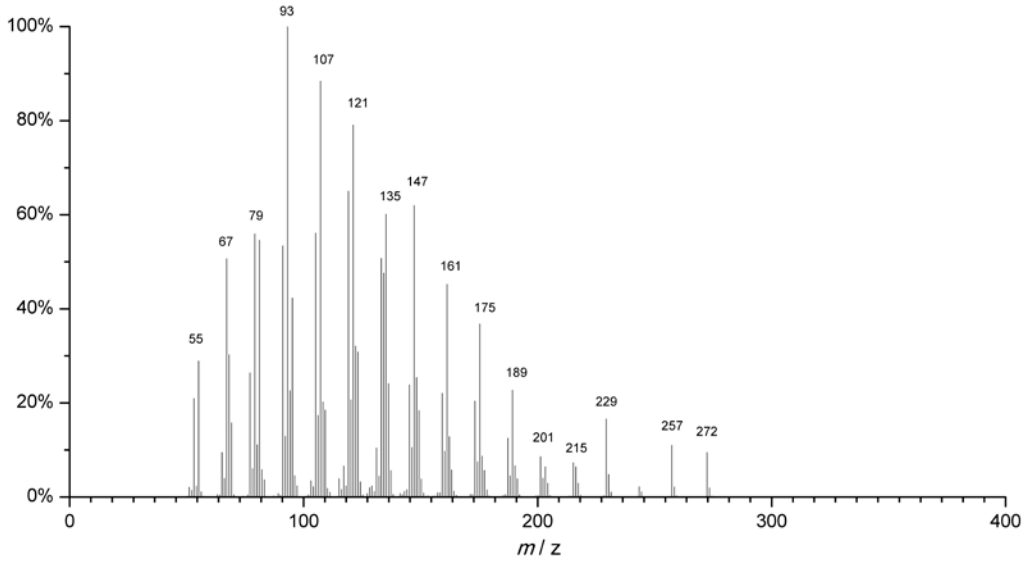

Figure S6 Mass spectrometry of the diterpene product dolabellatriene of CotB2^{W288G} (retention time 20.71 min), recorded on a Trace GC Ultra with DSQII (Thermo Scientific), *m/z* was analyzed from [50-650].