



**Supplementary information, Figure S6** Schematic diagram of the superposition between FabA and FabZ active tunnel. **(A)** Crystal structure of FabA-ACP complex (pdb code: 4KEH). Two FabA monomers and two ACP molecules are colored in white, blue, yellow and wheat, respectively. **(B)** Superposition of FabZ-ACP dimer subunit with FabA-ACP complex. **(C)** Active tunnel entrance area of FabZ-ACP and FabA-ACP complex. Gatekeeper residue (Arg104 in FabA, Tyr100 in FabZ) are shown in sticks. **(D)** Substrates in the active tunnel. The 8-carbon *trans*-2 fatty acid chain docked to ACP from FabZ-ACP complex and the 8-carbon *trans*-2 fatty acid crosslinked to FabA from FabA-ACP complex are shown as sticks, and colored in yellow and wheat respectively. **(E)** FabZ tunnel with 8-carbon *trans*-2 fatty acid chain is shaped by solid line. The 4'-phosphopantetheine arm, 8-carbon fatty acid chain, residues His23, Glu72, Gly78 and Ala153 from monomer B are colored in cyan, and the catalytic residue His58 from monomer A is colored in green. **(F)** The tunnel of FabA is shaped via solid line, and the FabZ tunnel is shaped by dashed line. Residues Pro29, Asn80, Val90, Val162 from FabA are colored in blue,

and the catalytic residue His70 is colored in orange. In FabA, the L-shape of the tunnel allows the reversion of the acyl chain carbonyl and adoption of a *cis*-3 conformation, while in FabZ, the fatty chain is accommodated in the U-shape tunnel and prevented the adoption of a *cis*-3 configuration.