Identification of residues important for the catalysis, structure maintenance, and substrate specificity of yeast 3-hydroxyacyl-CoA dehydratase Phs1

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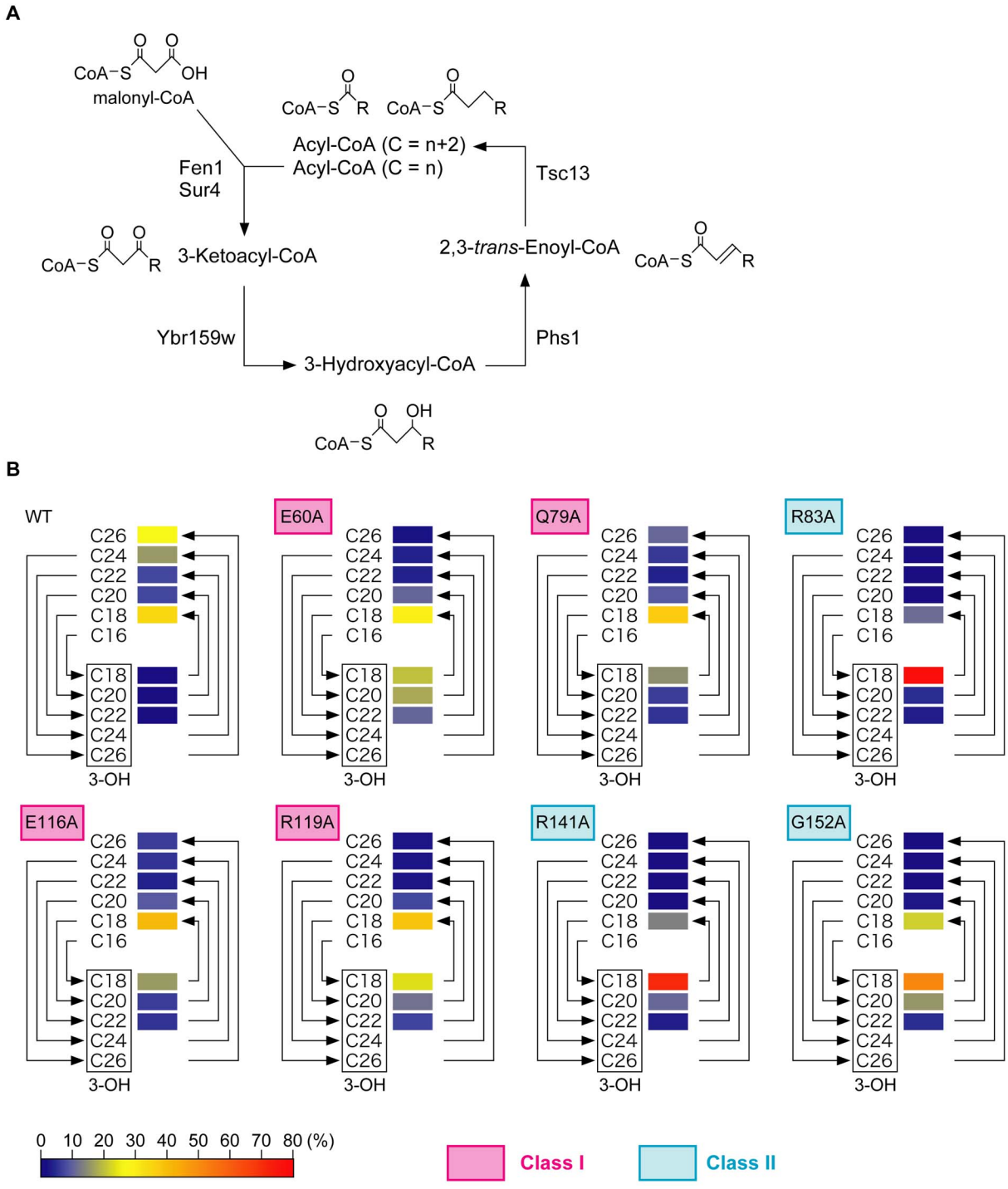

Fig. S1. FA elongation cycle in mutant membranes. (A) FA elongation cycle and enzymes responsible for each of the 4 reaction steps (1st step: fatty acid elongases Fen1/Sur4; 2nd step: 3-ketoacyl-CoA reductase Ybr159w; 3rd step: 3-hydroxyacyl-CoA dehydratase Phs1; and 4th step: 2,3-*trans*-enoyl-CoA reductase Tsc13). (B) Progression of the FA elongation cycle in mutant membranes. The yields of FAME and 3-hydroxy-FAME (Fig. 3B) are expressed as the percentage of the total products (FAMEs and 3-hydroxy-FAMEs) and are represented by colors. Note that the values for C18-FAME/3-hydroxy-C24 FAME and C20-FAME/3-hydroxy-C26-FAME were used as the values for C18-FAME and C20-FAME, respectively, assuming that the contribution of either 3-hydroxy-FAME was negligible.

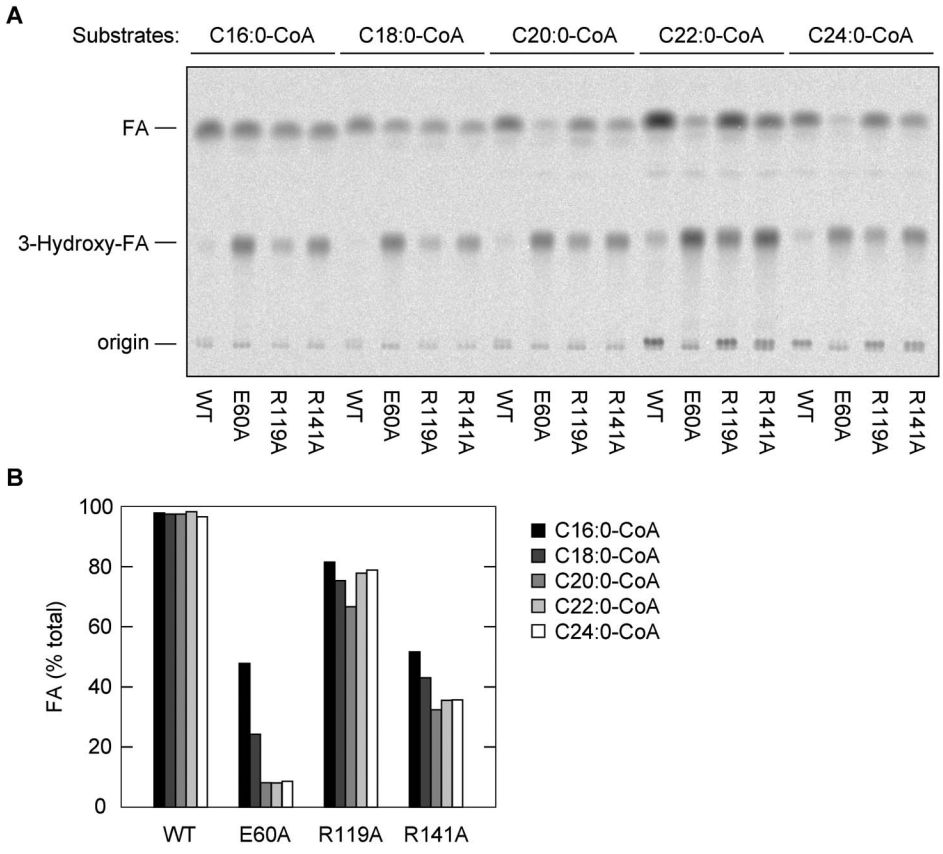

Fig. S2. Substrate specificity of the E60A mutant. (A and B) Total membrane fractions (20 μg) prepared from YTY19 (wild type; WT), YTY20 (E60A), YTY24 (R119A), and YTY25 (R141A) cells were incubated with 50 μM C16:0-, C18:0-, C20:0-, C22:0-, or C24:0-CoA and 27.3 μM [2-^{14}C]malonyl-CoA (0.075 μCi) for 30 min at 37 ˚C. Lipids were saponified, acidified, extracted, and separated by normal-phase TLC. Using a bioimaging analyzer BAS-2500, the radioactivity was detected (A) and quantified (B). Values represent percentages of acyl-CoA products relative to the total products (acyl-CoAs and 3-hydroxyacyl-CoAs).

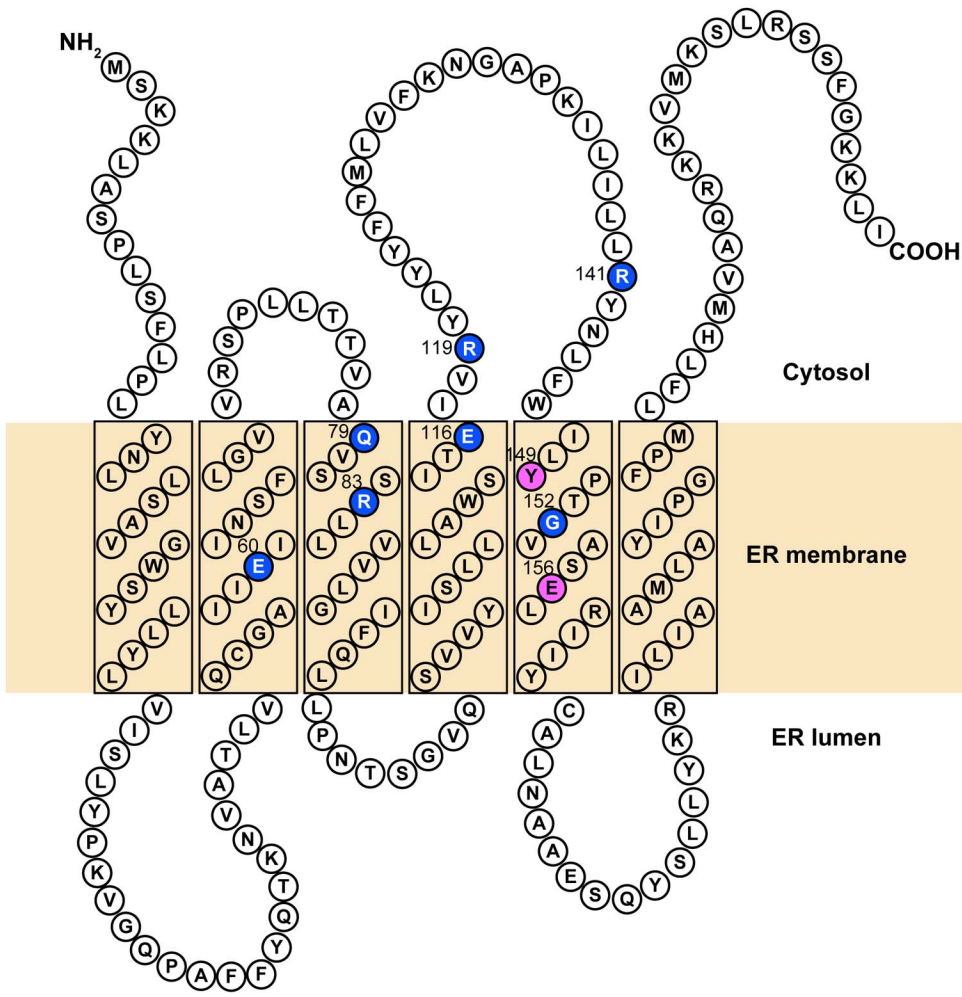

Fig. S3. Revised membrane topology model for Phs1. Our original model [1] was revised by changing the length of the transmembrane domain from 18 to 15 amino-acid residues, according to the report that yeast ER membrane proteins typically traverse the membrane via the hydrophobic stretch of 15 amino-acid residues [2]. Each of the 15 amino-acid residues in the six membrane spanning domains were selected to reflect a hydropathy plot [3] of Phs1. Blue and pink circles represent the mutated residues in this study and the putative catalytic residues identified in the previous investigation [1], respectively.

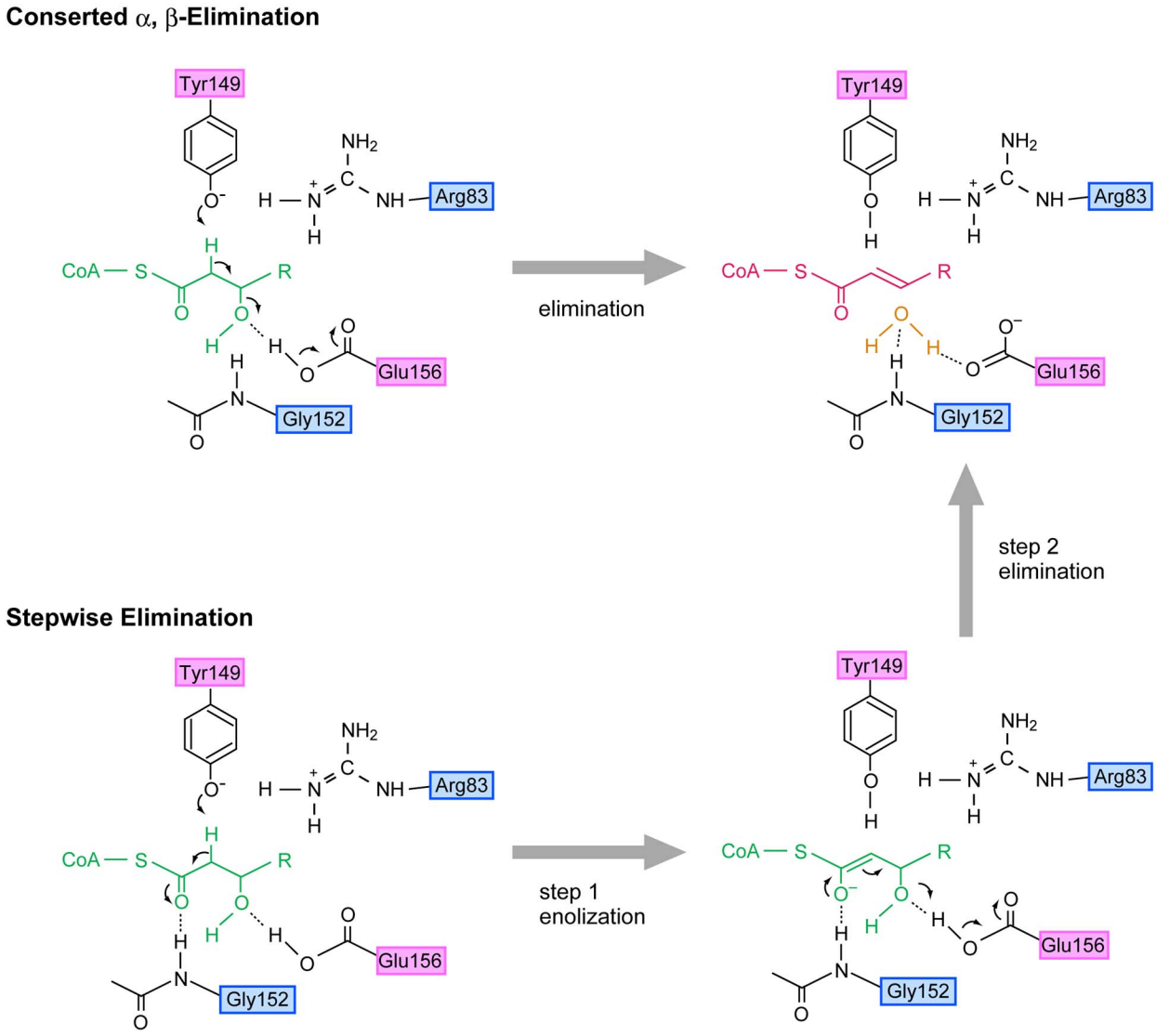

Fig. S4. Proposed catalytic mechanisms for Phs1. The original mechanism of the Phs1-catalyzed dehydration of 3-hydroxyacyl-CoA [1] was further refined to illustrate two possible mechanisms (a concerted and a stepwise elimination). The Tyr149 phenoxide, forming a salt bridge with the guanidinium group of the Arg83 residue, abstracts the α-hydrogen of 3-hydroxyacyl-CoA, and the Glu156 residue donates a proton to the β-hydroxyl group for its elimination as water. The Gly152 residue stabilizes the eliminated water through hydrogen bond interaction and may be also required for its removal from the active site. In the concerted α, β-elimination mechanism, the α-hydrogen abstraction and the elimination of the β-hydroxyl group occur simultaneously in a concerted manner. Alternatively, the stepwise elimination mechanism involves the initial formation of an enolate intermediate followed by dehydration. In the latter case, the Gly152 residue is probably facilitating the formation of the enolate intermediate through hydrogen bonding to the carbonyl oxygen as well as the enolate oxygen.

Supplemental references

[1] Kihara, A., Sakuraba, H., Ikeda, M., Denpoh, A. and Igarashi, Y. (2008) Membrane topology and essential amino acid residues of Phs1, a 3-hydroxyacyl-CoA dehydratase involved in very long-chain fatty acid elongation. J. Biol. Chem. 283, 11199-11209.

[2] Levine, T.P., Wiggins, C.A. and Munro, S. (2000) Inositol phosphorylceramide synthase is located in the Golgi apparatus of *Saccharomyces cerevisiae*. Mol. Biol. Cell 11, 2267-2281.

[3] Kyte, J. and Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157, 105-132.