Supplementary Information

Mechanism of Substrate Recognition and Insight into Feedback Inhibition of Homocitrate Synthase from *Thermus thermophilus*

Takuya Okada[‡1], Takeo Tomita[‡1], Asri Peni Wulandari[‡], Tomohisa Kuzuyama[‡], and Makoto Nishiyama[‡§2]

[‡]*Biotechnology Research Center, Department of Biotechnology, The University of Tokyo, Tokyo 113-8657, Japan,* [§]*RIKEN SPring-8 Center, Hyogo 679-5148, Japan*

Running title: Crystal structure of homocitrate synthase

[1]These authors contributed equally to this work.

[2]To whom correspondence should be addressed: Biotechnology Research Center, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
Tel.: +81-3-5841-3074; Fax: +81-3-5841-8030
E-mail: umanis@mail.ecc.u-tokyo.ac.jp

Legends for Supplementary Figures

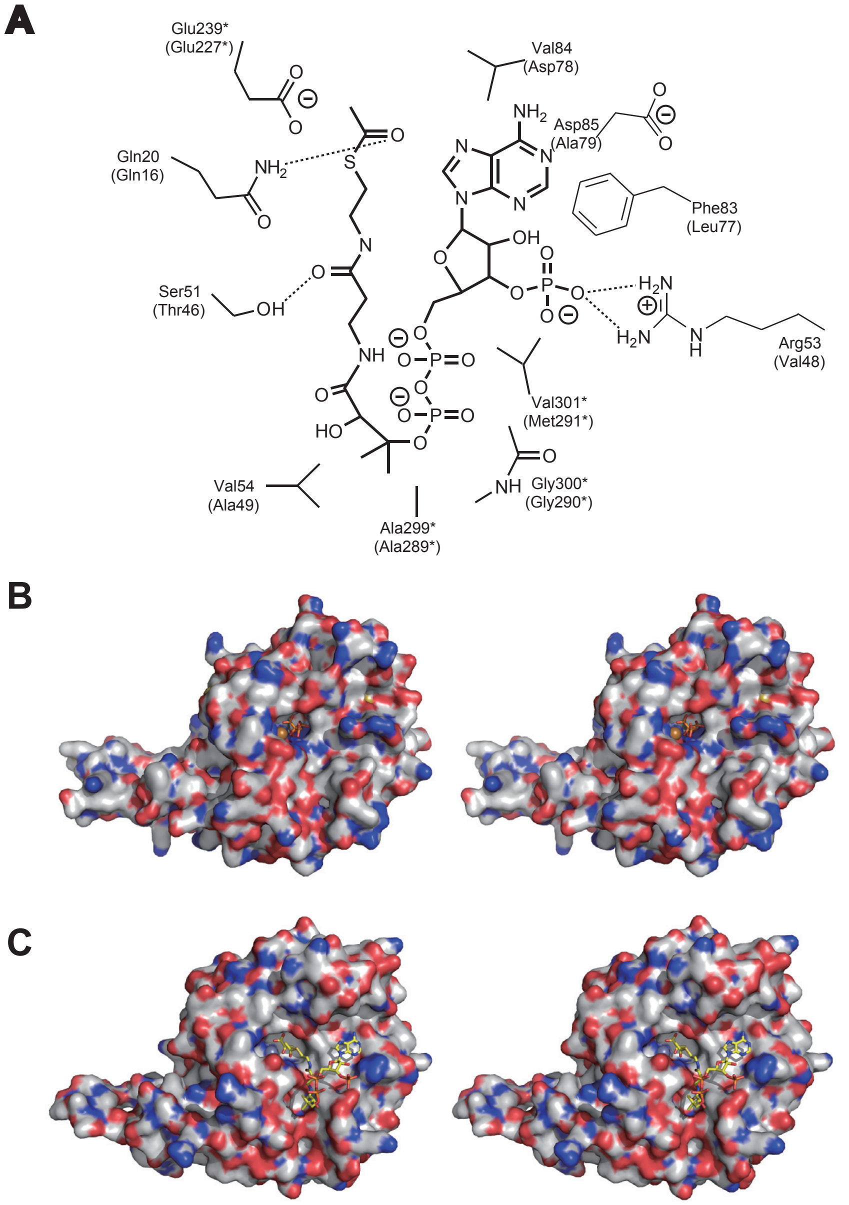
SUPPLEMENTARY FIGURE 1. **Comparison of acetyl-CoA binding residues between TtHCS and LiCMS.** *A*, Acetyl-CoA recognition of LiCMS is schematically illustrated. Residues responsible for acetyl-CoA recognition of LiCMS and residues located at the corresponding position in TtHCS (parentheses) are shown. Residues from adjacent subunit are indicated by asterisks. *B*, stereo view of the surfaces around the acetyl-CoA site of the LiCMS/Zn^{2+}/Pyr/acetyl-CoA complex; *C*, same view of the TtHCS/Cu^{2+}/α-KG complex. Carbon, oxygen, nitrogen, and sulfur atoms are in gray, red, blue, and yellow, respectively. Bound acetyl-CoA in LiCMS is shown with yellow sticks for carbon atoms. Pyruvate in the LiCMS/Zn^{2+}/Pyr/acetyl-CoA complex and α-KG in the TtHCS/Cu^{2+}/α-KG complex are shown by yellow and orange sticks. Cu^{2+} and Zn^{2+} ions in the TtHCS/Cu^{2+}/α-KG complex and

LiCMS/Pyr/acetyl-CoA complex are shown as orange and gray spheres, respectively.

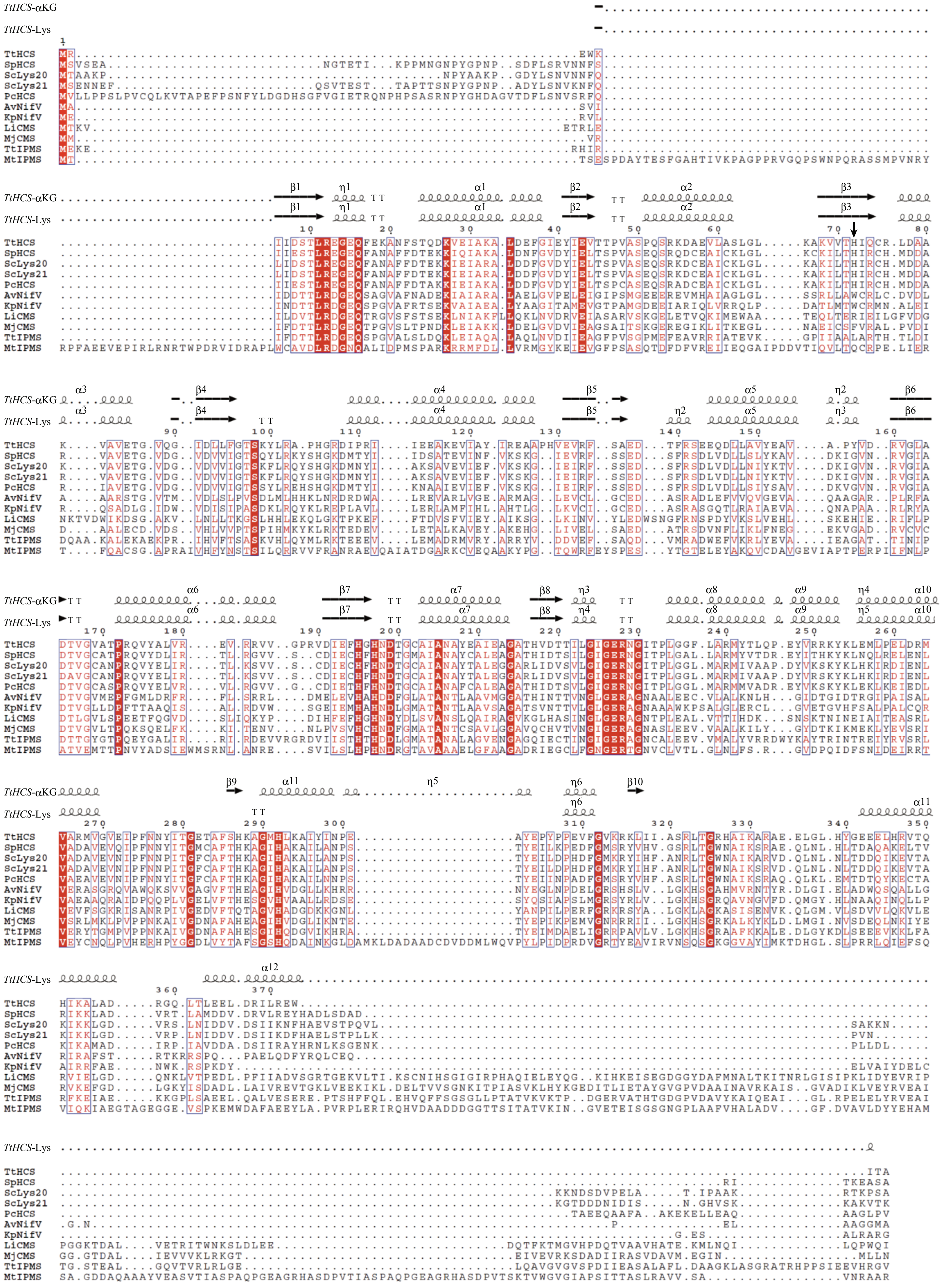
SUPPLEMENTARY FIGURE 2. **Amino acid sequence alignment among lysine-sensitive HCS, lysine-insensitive HCS (NifV), CMS, and IPMS.** Amino acid sequences were aligned by ClustalW (1) and alignment with secondary structures of TtHCS/Cu^{2+}/α-KG complex (*TtHCS*-αKG) and TtHCS/Co^{2+}/Lys complex (*TtHCS*-Lys) are drawn by ESPript (2). His72 of TtHCS is shown by vertical arrows. TtHCS, *T. thermophilus* HCS; SpHCS, *Schizosaccharomyces pombe* HCS; ScLys20, *Saccharomyces cerevisiae* HCS^{Lys20p}; ScLys21, *Saccharomyces cerevisiae* HCS^{Lys21p}; PcHCS, *Penicillium chrysogenum* HCS; AvNifV, *Azotobacter vinelandii* NifV; KpNifV, *Klebsiella pneumoniae* NifV; LiCMS, *L. interrogans* CMS; MjCMS, *M. jannaschii* CMS; TtIPMS, *T. thermophilus* IPMS; MtIPMS, *M. tuberculosis* IPMS.

References

1. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* 22, 4673-4680
2. Gouet, P., Courcelle, E., Stuart, D. I., and Mètoz, F. (1999) *Bioinformatics* 15, 305-308



Okada, T., et al., Supplementary Fig. 1



Okada, T., et al., Supplementary Fig. 2