

## Supplementary Information

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

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Running title: Crystal structure of homocitrate synthase

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### Legends for Supplementary Figures

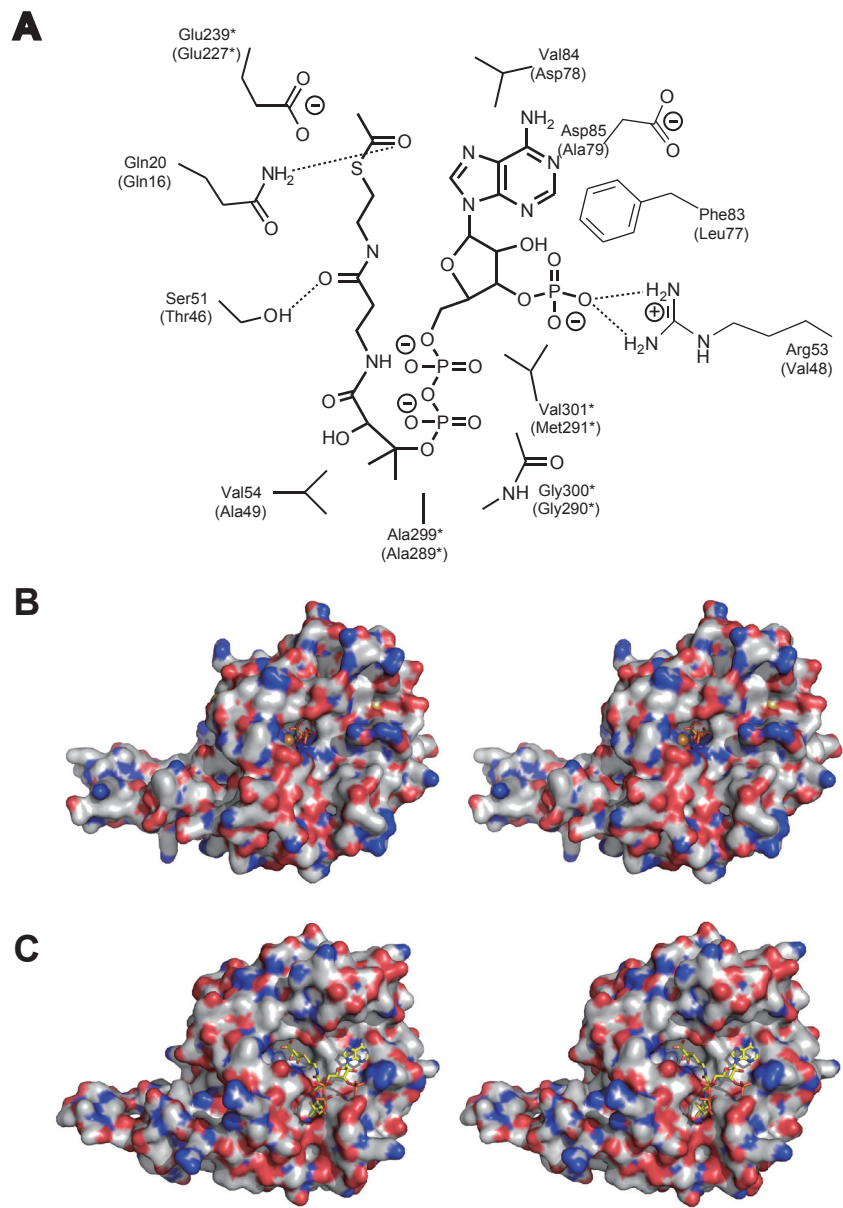
SUPPLEMENTARY FIGURE 1. **Comparison of acetyl-CoA binding residues between TtHCS and LiCMS.** *A*, Acetyl-CoA recognition of LiCMS is schematically illustrated. Residues responsible for acetyl-CoA recognition of LiCMS and residues located at the corresponding position in TtHCS (parentheses) are shown. Residues from adjacent subunit are indicated by asterisks. *B*, stereo view of the surfaces around the acetyl-CoA site of the LiCMS/Zn<sup>2+</sup>/Pyr/acetyl-CoA complex; *C*, same view of the TtHCS/Cu<sup>2+</sup>/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<sup>2+</sup>/Pyr/acetyl-CoA complex and α-KG in the TtHCS/Cu<sup>2+</sup>/α-KG complex are shown by yellow and orange sticks. Cu<sup>2+</sup> and Zn<sup>2+</sup> ions in the TtHCS/Cu<sup>2+</sup>/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<sup>2+</sup>/α-KG complex (*TtHCS-αKG*) and TtHCS/Co<sup>2+</sup>/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<sup>Lys20p</sup>; ScLys21, *Saccharomyces cerevisiae* HCS<sup>Lys21p</sup>; 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



TtHCS-αKG

TtHCS-Lys

TtHCS **M**R...  
 SpHCS **K**S...VSEEA...NGTETI...KPPMNGNPNYPGNP...SDFLSRVNNF...  
 ScLys20 **K**T...AAKP...NPYAAKP...GDYLSNVNMF...  
 ScLys21 **K**S...ENNEF...QSVTEST...TAPTTSNYPGNP...ADYLSNVKNF...  
 PcHCS **K**S...LLPSPSLPVCQLKVTAPFPPSNFYLDGGDHSGFVGIETRQNPHPASASRNYPYGHAGVTDFLSNVSRF...  
 AvNiFv **K**S...  
 KpNiFv **K**S...  
 LiCMS **M**K...KV...  
 MjCMS **M**M...  
 TtIPMS **M**M...KE...  
 MtIPMS **M**M...KE...TS...SPDAYTESFGAHTVVKPAGPPRRVQGPSWNPQRASSMPVNRV

TtHCS-αKG  $\beta$ 1  $\eta$ 1 TT  $\alpha$ 1  $\beta$ 2 TT  $\alpha$ 2  $\beta$ 3  $\eta$ 2  
 TtHCS-Lys  $\beta$ 1  $\eta$ 1 TT  $\alpha$ 1  $\beta$ 2 TT  $\alpha$ 2  $\beta$ 3  $\eta$ 2

TtHCS...  
 SpHCS...  
 ScLys20...  
 ScLys21...  
 PcHCS...  
 AvNiFv...  
 KpNiFv...  
 LiCMS...  
 MjCMS...  
 TtIPMS...  
 MtIPMS...

TtHCS-αKG  $\alpha$ 3  $\beta$ 4  $\alpha$ 4  $\beta$ 5  $\alpha$ 5  $\eta$ 2  $\beta$ 6  
 TtHCS-Lys  $\alpha$ 3  $\beta$ 4 TT  $\alpha$ 4  $\beta$ 5  $\eta$ 2  $\alpha$ 5  $\eta$ 3  $\beta$ 6

TtHCS...  
 SpHCS...  
 ScLys20...  
 ScLys21...  
 PcHCS...  
 AvNiFv...  
 KpNiFv...  
 LiCMS...  
 MjCMS...  
 TtIPMS...  
 MtIPMS...

TtHCS-αKG  $\alpha$ 6  $\beta$ 7  $\alpha$ 7  $\beta$ 8  $\eta$ 3  $\alpha$ 8  $\alpha$ 9  $\eta$ 4  $\alpha$ 10  
 TtHCS-Lys  $\alpha$ 6  $\beta$ 7 TT  $\alpha$ 7  $\beta$ 8  $\eta$ 4 TT  $\alpha$ 8  $\alpha$ 9  $\eta$ 5  $\alpha$ 10

TtHCS...  
 SpHCS...  
 ScLys20...  
 ScLys21...  
 PcHCS...  
 AvNiFv...  
 KpNiFv...  
 LiCMS...  
 MjCMS...  
 TtIPMS...  
 MtIPMS...

TtHCS-αKG  $\beta$ 9  $\alpha$ 11  $\eta$ 5  $\beta$ 10  $\alpha$ 11  
 TtHCS-Lys  $\beta$ 9 TT  $\alpha$ 11  $\beta$ 10  $\alpha$ 11

TtHCS...  
 SpHCS...  
 ScLys20...  
 ScLys21...  
 PcHCS...  
 AvNiFv...  
 KpNiFv...  
 LiCMS...  
 MjCMS...  
 TtIPMS...  
 MtIPMS...

TtHCS-Lys  $\alpha$ 12  
 TtHCS...  
 SpHCS...  
 ScLys20...  
 ScLys21...  
 PcHCS...  
 AvNiFv...  
 KpNiFv...  
 LiCMS...  
 MjCMS...  
 TtIPMS...  
 MtIPMS...

TtHCS-Lys...  
 TtHCS...  
 SpHCS...  
 ScLys20...  
 ScLys21...  
 PcHCS...  
 AvNiFv...  
 KpNiFv...  
 LiCMS...  
 MjCMS...  
 TtIPMS...  
 MtIPMS...