

Article

Structural exploration on palmitoyltransferase DHHC3 from *Homo sapiens*

Meng Tang ^{1,2}, Ying Xia ^{3,4}, Taoran Xiao ^{1,2}, Ruiyu Cao ^{1,2}, Yu Cao ^{3,4,*} and Bo OuYang ^{1,2,*}

- ¹ State Key Laboratory of Molecular Biology, Centre for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, China; tangmeng6@sibcb.ac.cn (M.T.); xiaotaoran2018@sibcb.ac.cn (T.X.); caoruiyu2018@sibcb.ac.cn (R.C.)
 - ² University of Chinese Academy of Sciences, Beijing 100049, China
 - ³ Institute of Precision Medicine, The Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 115 Jinzun Road, Shanghai 200125, China; xiaying@shsmu.edu.cn (Y.X.)
 - ⁴ Department of Orthopaedics, Shanghai Key Laboratory of Orthopaedic Implant, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, China
- * Correspondence: yu.cao@shsmu.edu.cn (Y.C.); ouyang@sibcb.ac.cn (B.O.)

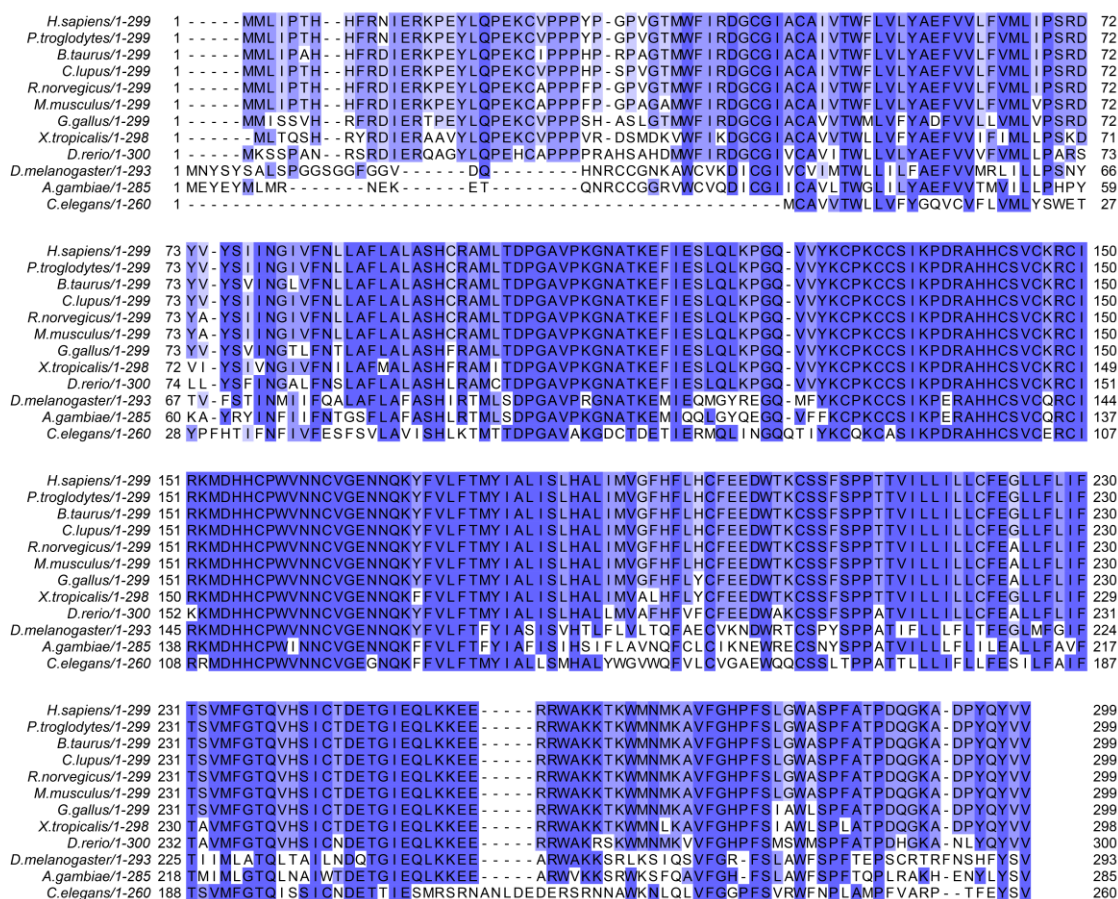


Figure S1. Sequence alignment of DHHC3 homologs. Amino acid sequences of DHHC3 homologs were aligned by Clustal Omega [37]. Species contain *Homo sapiens* (human), *Pan troglodytes* (chimpanzee), *Bos taurus* (bovine), *Canis lupus* (wolf), *Rattus norvegicus* (rat), *Mus musculus* (mouse), *Gallus gallus* (Chicken), *Xenopus tropicalis* (clawed frog), *Danio rerio* (zebrafish), *Drosophila melanogaster* (fruit fly), *Anopheles gambiae* (mosquito), *Caenorhabditis elegans*.

Table S1. Sequence identity matrix of mammalian DHHC3

	<i>H. sapiens</i>	<i>P. troglodytes</i>	<i>C. lupus</i>	<i>R. norvegicus</i>	<i>M. musculus</i>	<i>B. taurus</i>
<i>Homo sapiens</i>	100	100	99	98.33	97.32	97.32
<i>Pan troglodytes</i>	100	100	99	98.33	97.32	97.32
<i>Canis lupus</i>	99	99	100	98.33	97.32	97.99
<i>Rattus norvegicus</i>	98.33	98.33	98.33	100	99	96.99
<i>Mus musculus</i>	97.32	97.32	97.32	99	100	96.66
<i>Bos taurus</i>	97.32	97.32	97.99	96.99	96.66	100

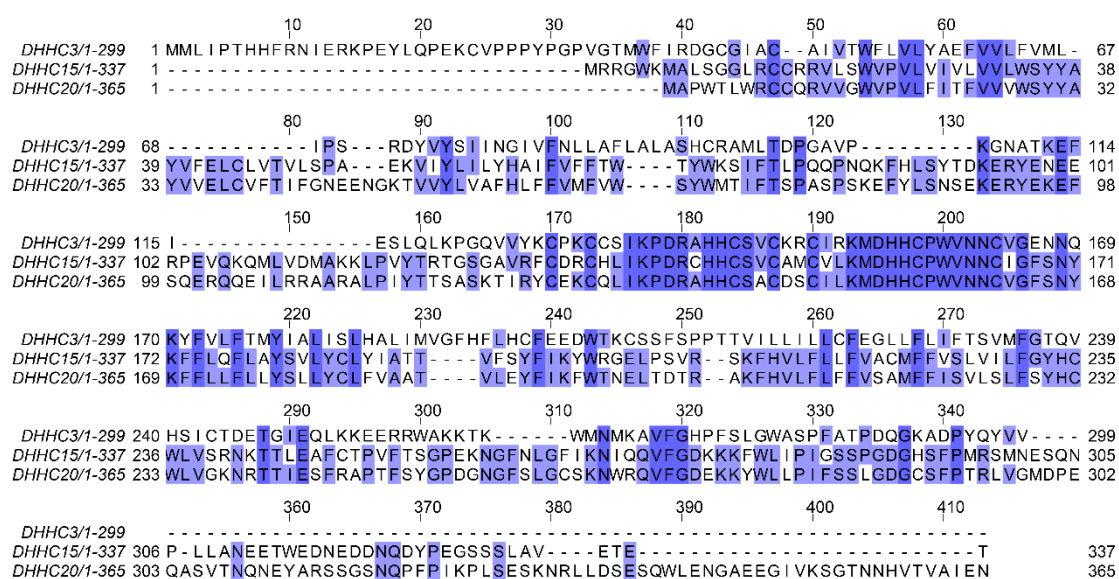


Figure S2. Sequence alignment of DHHC3, DHHC15 and DHHC20. Amino acid sequences of human DHHC3, DHHC15 and DHHC20 were aligned by Clustal Omega [37]. DHHC3 shows low sequence identity with DHHC15 (25.4 %) and DHHC20 (29.5 %), while CRD including DHHC motif is highly conserved in these 3 members.

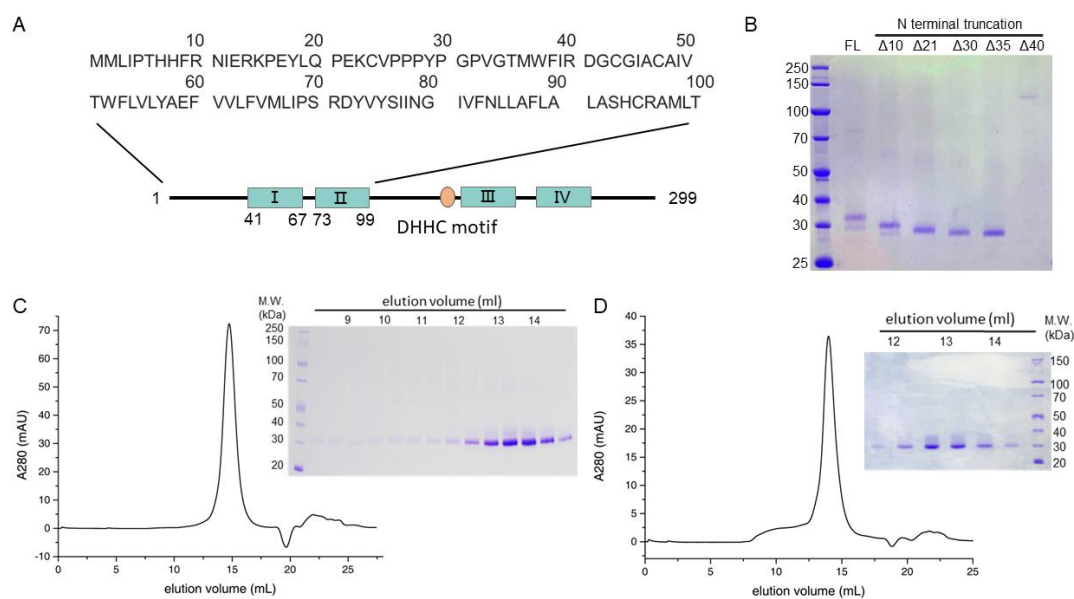


Figure S3. Expression and purification of truncated hDHH3s. (A) Topological scheme and N-terminal sequence of hDHH3. (B) Expression test of hDHH3 with different N-terminal truncations. (C) Size exclusion chromatography and SDS-PAGE of hDHH3-Δ21 and (D) hDHH3-Δ35.

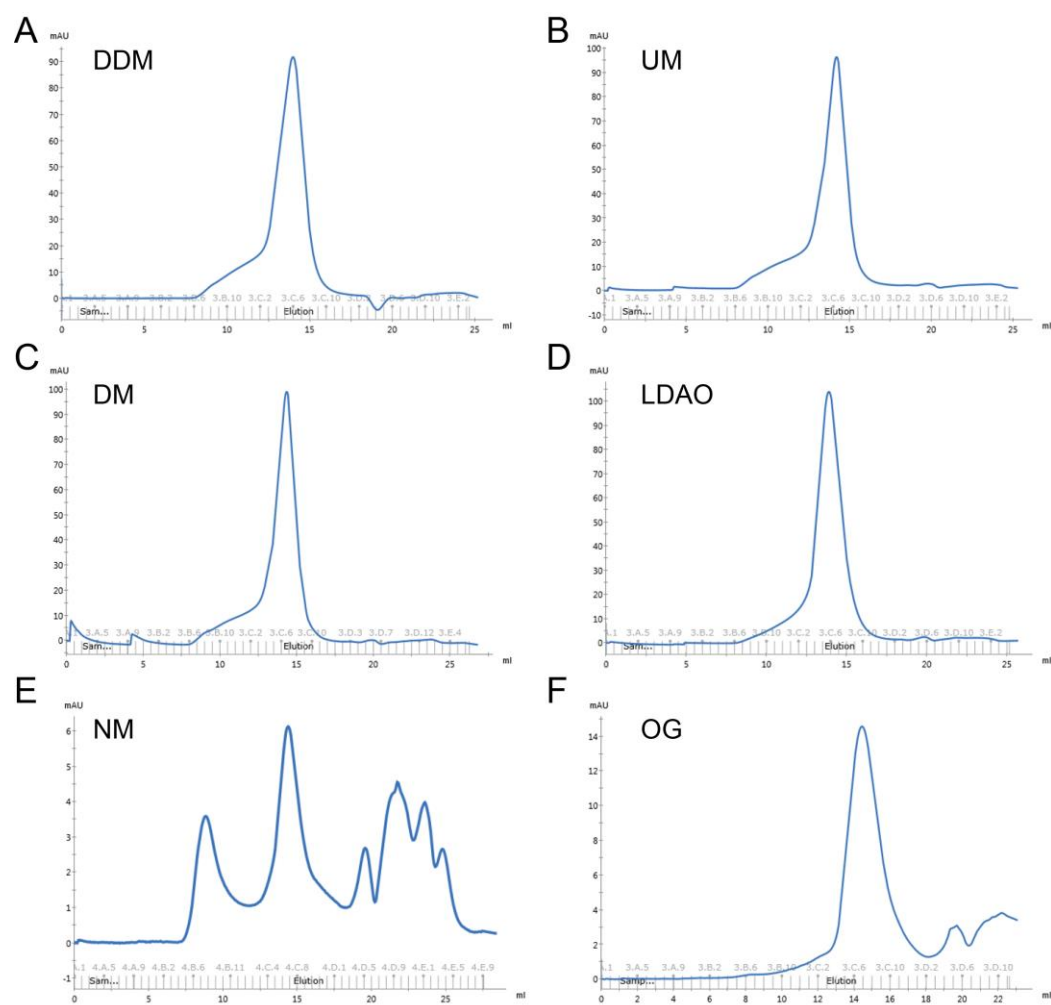


Figure S4. Detergent screening of hDHHC3-Δ21. Size exclusion chromatography profiles of hDHHC3-Δ21 in FPLC buffer containing 25 mM HEPES, 150 mM NaCl with (A) 1 mM DDM, (B) 2 mM UM, (C) 4 mM DM, (D) 0.15 % LDAO, (E) 12 mM NM and (F) 40 mM OG, respectively.

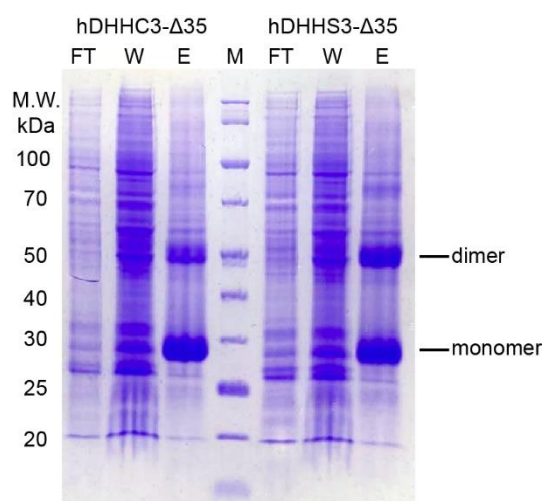


Figure S5. Dimerization of hDHHC3-Δ35 and hDHHS3-Δ35. An obvious dimeric band of hDHHS3-Δ35 was observed on the SDS-PAGE gel.

Reference

37. Madeira, F.; Pearce, M.; Tivey, A.R.N.; Basutkar, P.; Lee, J.; Edbali, O.; Madhusoodanan, N.; Kolesnikov, A.; Lopez, R. Search and sequence analysis tools services from EMBL-EBI in 2022. *Nucleic Acids Res.* **2022**, doi:10.1093/nar/gkac240.