SUPPLEMENTARY FIGURES

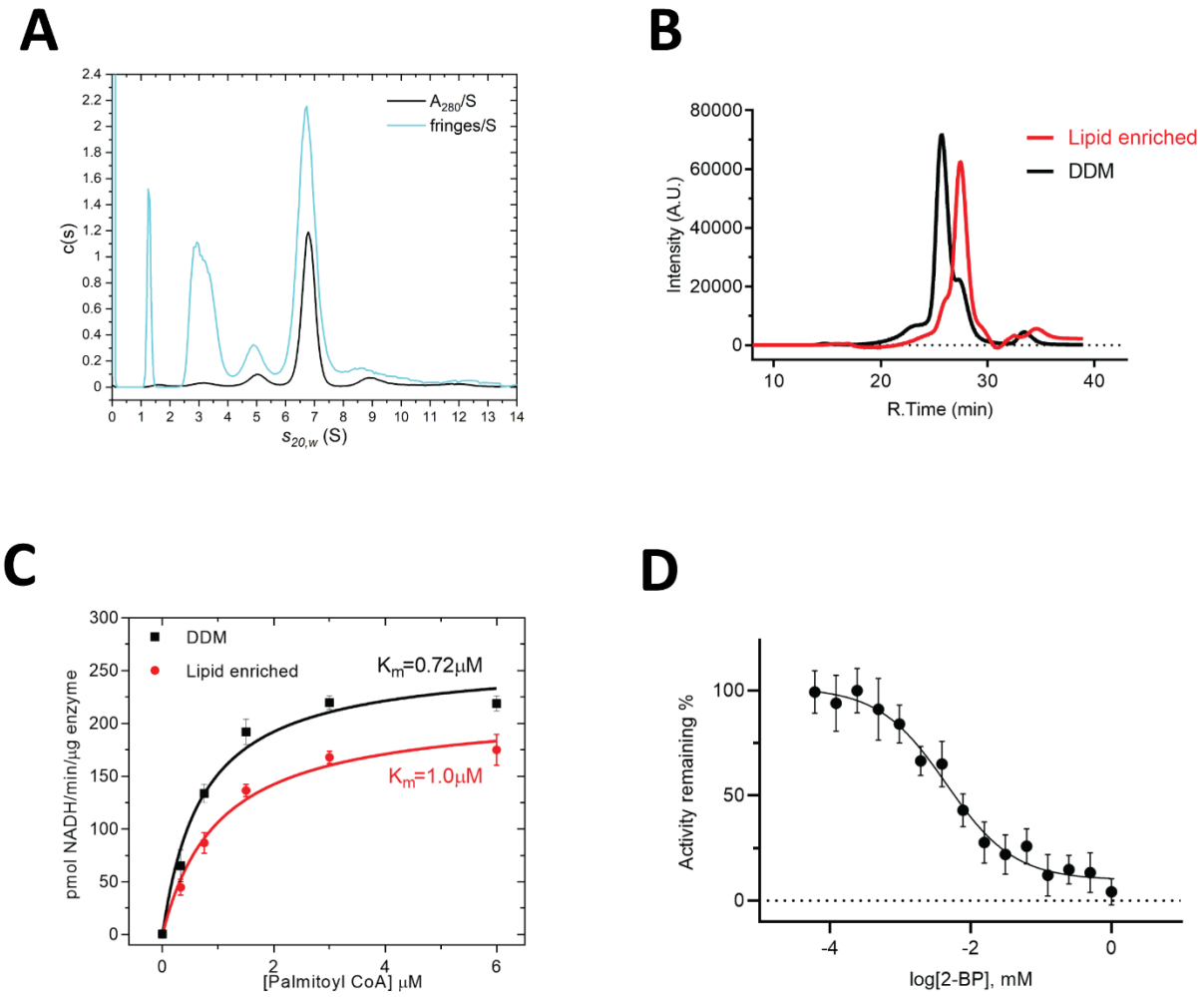

Figure S1. Monomeric state of DHHC20 in a lipid-rich environment. **(A)** Sedimentation velocity experiments of the detergent solubilized DHHC20. The absorbance and interference c(*s*) distributions were analyzed in GUSSI. The integration of the major species results in a species at 6.77 ± 0.02 S with a molar mass of 170 ± 30 kDa, of which 80 ± 13 kDa represents the protein. **(B)** FSEC profiles for mVneus-DHHC fusion protein purified in DDM or lipid-rich conditions. **(C)** Determination of kinetic parameters for the autopalmitoylation of hDHHC20 using the coupled-enzyme assay. Shown is a Michaelis-Menten fit to palmitoyl CoA titration with hDHHC20 purified in DDM (black square) or lipid-rich conditions (red circle), with Km=0.72±0.1 µM (kcat=17.4±1 min^{-1}) and Km=1.0±1 µM (kcat=14.3±0.8 min^{-1}), respectively. **(D)** IC_{50} curve for the inhibition of hDHHC20 purified in lipid-rich conditions by 2-bromopalmitate (2-BP). IC_{50} value (4.5 uM) were determined by fitting a three-parameter dose–response curve (GraphPad Prism); n 3; error bars, S.D.

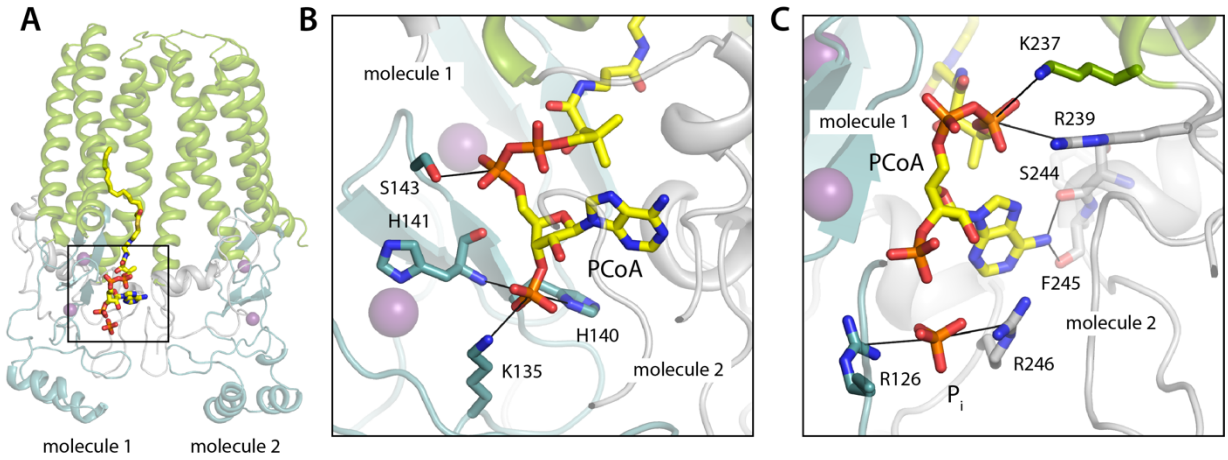

Figure S2. Unit cell of the hDHHS20-PCoA crystal. (A) The unit cell contains two hDHHC molecules (cartoons); a complete palmitoyl-CoA (sticks) can be discerned bound to molecule 1. (B) Polar contacts between the headgroup of palmitoyl-CoA and molecule 1. (C) Polar contacts between the headgroup of palmitoyl-CoA and molecule 2. A phosphate ion (P_i) from the buffer binds in close proximity to the terminal phosphate group of PCoA, and is coordinate by residues in molecule 1.

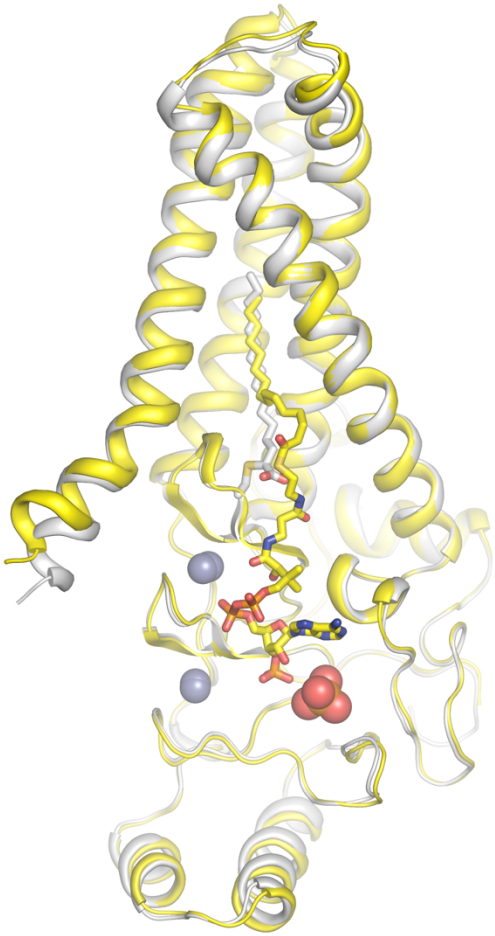

Figure S3. Superposition of the 2-BP complex of hDHHC20 (9) with the crystal structure of hDHHS20-PCoA complex. The 2-BP structure is shown in light grey and the hDHHS20-PCoA structure ios shown in yellow. The zinc ions are shown as grey spheres.

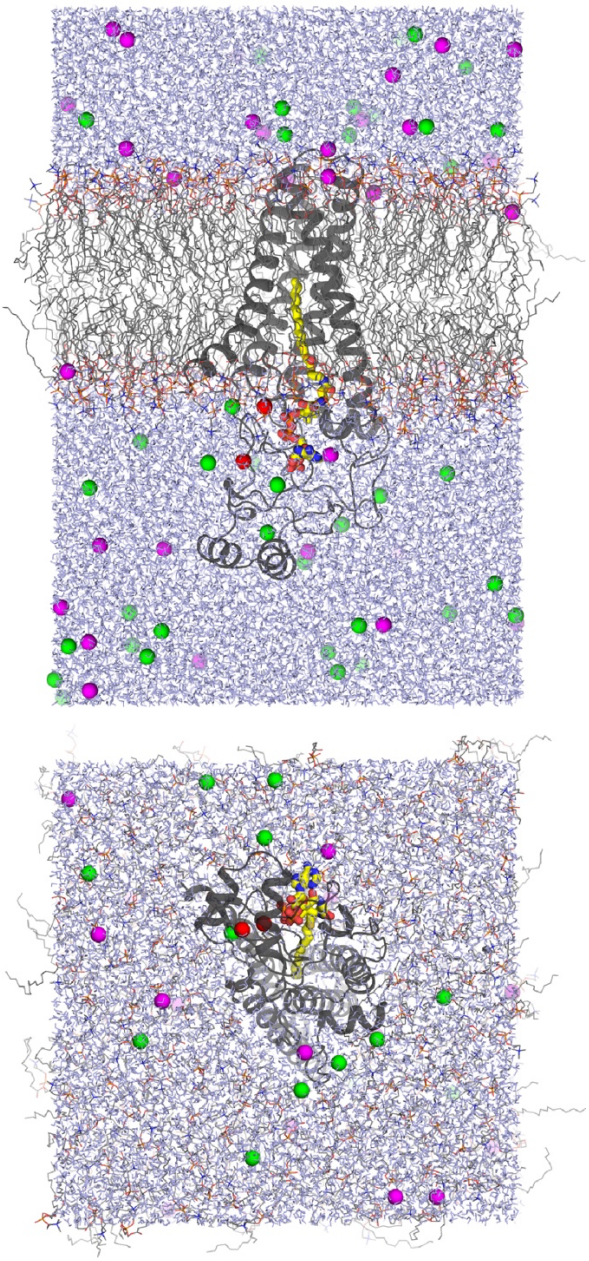

Figure S4. Molecular dynamics simulation of hDHHC20 with bound palmitoyl-CoA. The figure shows a snapshot of one of the molecular systems considered in the all-atom simulations reported in this study, viewed along the membrane plane (*upper panel*) and from the cytoplasmic space (*lower panel*). This system consists of wild-type hDHHC20 (*gray cartoon*) with bound Zn^{2+} (*red spheres*); a palmitoyl-CoA molecule (*sticks*); a POPC lipid bilayer (*lines*); and a solution containing 100 mM NaCl plus counterions neutralizing the total system charge (green/magenta/purple). All boundaries are periodic.

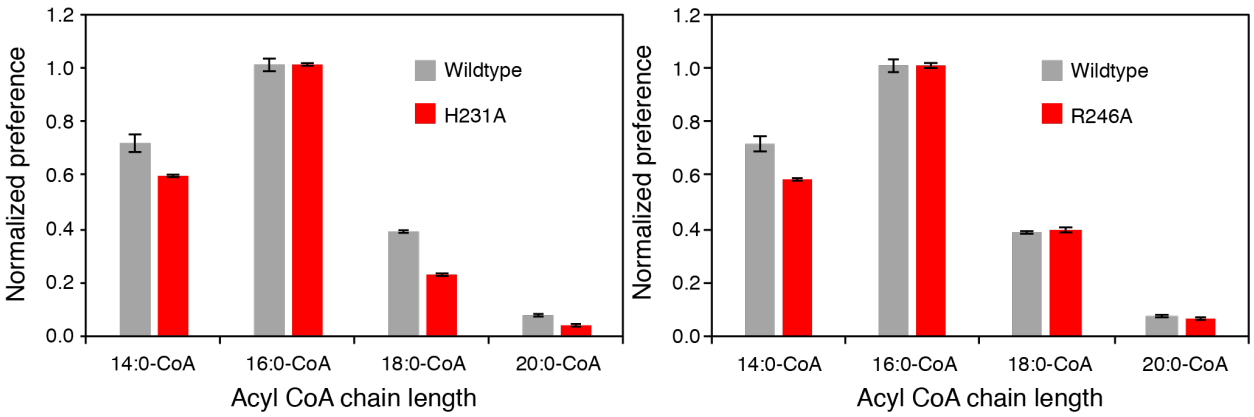

Figure S5. Acyl-CoA chain–length selectivity of mutants of residues that contact PCoA headgroup. As determined by the autoacylation assay. The x axis shows the carbon-chain lengths of different acyl-CoA donors, and the y axis shows normalized activity (initial velocity) of wild-type or mutant versions of hDHHC20. Each data set is individually normalized to 1 for the activity with regard to palmitoyl-CoA.

Table S1. Data collection and refinement statistics.

	DHHS_160CoA
Wavelength	
Resolution range	67.53 - 2.88 (2.983 - 2.88)
Space group	P 1 21 1
Unit cell	53.529 114.61 83.596 90 91.234 90
Total reflections	160670 (16119)
Unique reflections	22872 (1798)
Multiplicity	6.9 (7.0)
Completeness (%)	96.90 (78.10)
Mean I/sigma(I)	7.81 (0.59)
Wilson B-factor	92.26
R-merge	0.1685 (3.203)
R-meas	0.1823 (3.461)
R-pim	0.06881 (1.302)
CC1/2	0.991 (0.503)
CC*	0.998 (0.818)
Reflections used in refinement	22196 (1797)
Reflections used for R-free	1974 (165)
R-work	0.2783 (0.5536)
R-free	0.3283 (0.6430)
CC(work)	0.906 (0.748)
CC(free)	0.881 (0.435)
Number of non-hydrogen atoms	4702

	4583
macromolecules	
ligands	105
solvent	14
Protein residues	586
RMS(bonds)	0.003
RMS(angles)	0.56
Ramachandran favored (%)	97.38
Ramachandran allowed (%)	2.62
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.45
Clashscore	1.92
Average B-factor	129.99
	129.76
macromolecules	
ligands	142.11
solvent	112.28

Statistics for the highest-resolution shell are shown in parentheses.

Table S1. Data collection and refinement statistics for the crystal structure of human DHHS20 with palmitoyl CoA