Regulated degradation of HMG CoA reductase requires conformational changes in sterol-sensing domain

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3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) is the rate-limiting enzyme in cholesterol synthesis and target of cholesterol-lowering statin drugs. Accumulation of sterols in endoplasmic reticulum (ER) membranes accelerates degradation of HMGCR, slowing the synthesis of cholesterol. Degradation of HMGCR is inhibited by its binding to UBIAD1 (UbiA prenyltransferase domain-containing protein-1). This inhibition contributes to statin-induced accumulation of HMGCR, which limits their cholesterol-lowering effects. Here, we report cryo-electron microscopy structures of the HMGCR-UBIAD1 complex, which is maintained by interactions between transmembrane helix (TM) 7 of HMGCR and TMs 2–4 of UBIAD1. Disrupting this interface by mutagenesis prevents complex formation, enhancing HMGCR degradation. TMs 2–6 of HMGCR contain a 170-amino acid sterol sensing domain (SSD), which exists in two conformations—one of which is essential for degradation. Thus, our data supports a model that rearrangement of the TMs in the SSD permits recruitment of proteins that initiate HMGCR degradation, a key reaction in the regulatory system that governs cholesterol synthesis.

3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) is a polytopic, endoplasmic reticulum (ER)-localized glycoprotein that catalyzes a rate-limiting step in synthesis of cholesterol and essential nonsterol isoprenoids such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate (GGpp). HMGCR is tightly controlled by a complex feedback regulatory system that allows cells to constantly synthesize nonsterol isoprenoids while avoiding toxic overproduction of cholesterol and other sterols. Part of this feedback control involves accelerated ERAD (ER-associated degradation) of HMGCR. This ERAD is initiated by the accumulation of sterols in ER membranes, which triggers binding of HMGCR to ER membrane proteins called Insigs. Insig binding is mediated by the N-terminal membrane domain of HMGCR, which is both necessary and sufficient for ERAD and contains eight transmembrane helices (TMs) that precede a large cytosolic catalytic domain. TMs 2–6 of HMGCR comprise what is known as the sterol-sensing domain (SSD); mutation of a tetrapeptide sequence (Y75IYF) in the SSD of HMGCR abolishes its binding to Insigs, preventing ubiquitination and ERAD. Insig-associated ubiquitin ligases mediate ubiquitination of lysines-89 and −248 (K89 and K248), which are exposed to the cytosol and lie adjacent to TMs 3 and 7 of HMGCR, respectively. Sterol-induced ubiquitination marks HMGCR for extraction across ER membranes, after which it becomes dislocated into the cytosol for proteasomal degradation. The combination of K89R and K248R mutations prevent sterol-induced ubiquitination and ERAD of HMGCR in both cultured cells and tissues of knock-in mice.

Whereas Insigs accelerate the ERAD of HMGCR, another protein called UBIAD1 (UbiA prenyltransferase domain-containing protein-1)
binds to and stabilizes the enzyme. We subsequently identified UBIAD1 as a GGpp sensor that binds to HMGCR and inhibits its ERAD when ER membranes are depleted of GGpp. When GGpp accumulates within ER membranes, the isoprene binds to UBIAD1, causing it to dissociate from HMGCR and translocate to the medial-trans cisternae of the Golgi. Importantly, GGpp-induced translocation of UBIAD1 from the ER-to-Golgi occurs in HMGCR-deficient cells. Dissociation from UBIAD1 allows for the maximal ERAD of HMGCR (Fig. 1a). The physiologic significance of the UBIAD1-HMGCR interaction is confirmed by the observation that missense mutations in UBIAD1 cause Schnyder corneal dystrophy (SCD), an autosomal-dominant eye disease characterized by corneal opacification owing to the over-accumulation of cholesterol. SCD-associated variants of UBIAD1 are sequestered in the ER and resist GGpp-induced dissociation from HMGCR (Fig. 1a). As a result, SCD-associated UBIAD1 inhibits ERAD of HMGCR, which leads to enhanced synthesis and accumulation of cholesterol in both cultured cells and tissues of mice. Competitive inhibitors of HMGCR called statins are prescribed to lower circulating levels of low density lipoprotein (LDL)-cholesterol and reduce the incidence of atherosclerotic cardiovascular disease (ACVD). However, the efficacy of statins is reduced because they disrupt feedback control of HMGCR owing to depletion of sterol and nonsterol isoprenoids (including GGpp). This depletion leads to the

![Fig. 1](https://example.com/fig1.png)

**Fig. 1** Cryo-EM structure of the HMGCR-UBIAD1 complex. a Overview of UBIAD1-mediated regulation of HMGCR ERAD. b Schematic representation of HMGCR and UBIAD1 variants used for structural determination. The position of BRIL insertion in UBIAD1 is indicated. c Cryo-EM map of HMGCR-UBIAD1 complex 1. d Overall structure of HMGCR-UBIAD1 complex 1 viewed from the side of the membrane (left) and cytosol (right). The TMs of UBIAD1 are denoted by underlining. The cartoon denotes a slice of the TMs of HMGCR and UBIAD1 with helices indicated by numbers. e Cryo-EM map of HMGCR-UBIAD1 complex 2a. f Overall structure of HMGCR-UBIAD1 complex 2a and complex 2b viewed from the membrane side.
accumulation of HMGCR in the liver that overcomes inhibitory effects of statins, allowing continued synthesis of cholesterol that limits lowing of plasma cholesterol. Our previous studies indicated that inhibition of ERAD substantially contributes to statin-induced accumulation of HMGCR in the liver. Here, we determined cryogenic electron microscopy (cryo-EM) structures of HMGCR bound to SCD-associated UBIAD1 (N102S). Structural and functional analysis provide key insights into mechanisms for UBIAD1-mediated protection of HMGCR from ERAD. These findings have important implications for development of agents that enhance statin efficacy and reduce ACVD. Moreover, our studies reveal that the HMGCR SSD adopts a specific conformation required for sterol-accelerated ERAD, establishing the molecular basis through which the region mediates regulation of cholesterol synthesis.

Results

Assembly of a complex between HMGCR and UBIAD1

We focused on the stabilizing interaction between the membrane domain of hamster HMGCR and UBIAD1. The hamster proteins share over 95% overall identity with their human counterparts (Supplementary Fig. 1). Expression plasmids were prepared that encode the FLAG-tagged membrane domain of HMGCR harboring arginine substitutions for K89 and K248 (designated HMGCR) and Strep-tagged UBIAD1 from which we deleted a flexible N-terminal region (amino acids 1–40). We included the SCD-associated N102S mutation, which blunts enzymatic activity; this protein is designated UBIAD1 (Fig. 1b). Notably, UBIAD1 containing the N-terminal deletion continued to localize to the Golgi of Gpp-replete cells (Supplementary Fig. 2), indicating the protein was normally folded. HMGCR and UBIAD1 were co-expressed in HEK-293 GnpT1 cells and purified by anti-FLAG chromatography. Gel filtration shows that the HMGCR-UBIAD1 complex migrated as a single peak (Supplementary Fig. 3a); the presence of both proteins in the peak fraction was confirmed by immunoblot and mass spectrometry. Unfortunately, cryo-EM images of the HMGCR-UBIAD1 complex displayed limited features and we failed to reconstitute a 3D model.

Hydropathy plots predict that UBIAD1 is comprised of 9 TMs. To provide a fiducial marker for particle image alignment in cryo-EM structure determination, we inserted the soluble, thermostabilized apocytochrome b562RIL (BRIL) in a cytosolic loop predicted to localize to the Golgi of Gpp-replete cells (Supplementary Fig. 2), indicating the protein was normally folded. HMGCR and UBIAD1 were co-expressed in HEK-293 GnpT1 cells and purified by anti-FLAG chromatography. Gel filtration shows that the HMGCR-UBIAD1 complex migrated as a single peak (Supplementary Fig. 3a); the presence of both proteins in the peak fraction was confirmed by immunoblot and mass spectrometry. Unfortunately, cryo-EM images of the HMGCR-UBIAD1 complex displayed limited features and we failed to reconstitute a 3D model.

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To address this problem, we generated and screened ~1000 hybridoma clones for conformation-specific antibodies using HMGCRTM, UBIAD1N102S complex as antigen. We identified one monoclonal antibody designated IgG-15B2 that bound native HMGCRTM-UBIAD1N102S, and found that BRIL insertion did not interfere the epitope recognition. Fab<sub>B</sub>, a Fab fragment derived from IgG-15B2, was co-purified with HMGCRTM-UBIAD1-BRIL<sub>N102S</sub> upon gel filtration (Supplementary Fig. 3b, e).

The structure of the HMGCRTM<sub>1</sub>-UBIAD1-BRIL<sub>N102S</sub>-Fab<sub>B</sub> complex was determined by cryo-EM at a resolution of 3.3-Å (Fig. 1c, e, Supplementary Table 1). HMGCRTM<sub>1</sub>-UBIAD1-BRIL<sub>N102S</sub>-Fab<sub>B</sub> complex existed as either a monomeric or dimeric heterotrimer. We observed clear densities for all 9 TMs of UBIAD1 and TMs 1-7 of HMGCR in the monomeric UBIAD1-HMGCR complex 1) (Fig. 1c, d, Supplementary Figs. 5, 6).

Interestingly, within the dimeric structure of the HMGCRTM-UBIAD1-BRIL<sub>N102S</sub>-Fab<sub>B</sub> complex, structural analysis revealed the UBIAD1-HMGCR interface was identical in both complexes (Supplementary Figs. 4 and 9c).

The conformation of UBIAD1 is identical in Complexes 1 and 2a (Fig. 2a). The interface between UBIAD1 and HMGCR occupies an area of ~1000 Å<sup>2</sup>; the complex is maintained by several interactions between TMs 2 and 4 of UBIAD1 and TMs 5 and 7 of HMGCR (Figs. 1d and 2a). The interaction of residues in TM5 and TM7 of HMGCR with residues in TM4 and TM2 of UBIAD1 (Fig. 2b, c).

Overall Structure of the UBIAD1-HMGCR complex
Cryo-EM maps revealed that the Fab<sub>B</sub> epitope encompasses the cytosolic interface of the HMGCRTM<sub>1</sub>-UBIAD1-BRIL<sub>N102S</sub> complex and includes regions of both proteins (Fig. 1c, e, Supplementary Fig. 8). The possibility exists that Fab<sub>B</sub> modulates complex formation by altering the structure of HMGCRTM<sub>1</sub> and/or UBIAD1-BRIL<sub>N102S</sub>. Structural analysis of HMGCRTM<sub>1</sub>-UBIAD1-BRIL<sub>N102S</sub>-Fab<sub>B</sub> indicates that Fab<sub>B</sub> and Nb disrupted the dimer interface (Supplementary Fig. 9a, b); thus, the HMGCRTM<sub>1</sub>-UBIAD1-BRIL<sub>N102S</sub>-Fab<sub>B</sub>-Nb complex was only observed in the monomeric state. Although the overall resolution of HMGCRTM<sub>1</sub>-UBIAD1-BRIL<sub>N102S</sub>-Fab<sub>B</sub>-Nb complex is lower than that of the HMGCRTM<sub>1</sub>-UBIAD1-BRIL<sub>N102S</sub>-Fab<sub>B</sub>-Nb complex, structural analysis revealed the UBIAD1-HMGCR interface was identical in both complexes (Supplementary Figs. 4 and 9c).

We next compared the association of UBIAD1<sub>N102S</sub> with HMGCRTM<sub>1</sub> and variants of the protein harboring mutations in the HMGCRTM<sub>1</sub>-UBIAD1 interface predicted to disrupt complex formation (V181R, V252R, V256R, and V267R in HMGCR) (Fig. 2b, c). Because the HMGCRTM<sub>1</sub>-UBIAD1 complex cannot be assembled in vitro, we used co-immunoprecipitation of the proteins expressed in vivo to measure their association. HEK-293 GnTI cells transfected with expression plasmids encoding Strep-tagged UBIAD1<sub>N102S</sub> and FLAG-tagged HMGCRTM<sub>1</sub> or its variants were lysed and precipitated with anti-FLAG.
coupled agarose beads. Immunoblot analysis of precipitated material revealed that as expected, UBIAD1N102S co-precipitated with HMGCRTM (Fig. 2d, lane 3). In contrast, UBIAD1N102S failed to be co-precipitated with HMGCRTM (V252R) and HMGCRTM (V256R) (lanes 5 and 6); HMGCRTM (V181R) and HMGCRTM (V267R) precipitated UBIAD1N102S albeit at slightly reduced levels compared to HMGCRTM (lanes 4 and 7). We also generated a variant of UBIAD1N102S containing a point mutation (F172A) at the HMGCR-UBIAD1 complex interface (Fig. 2b, c). The results show that co-precipitation of UBIAD1N102S (F172A) with HMGCRTM was reduced compared to UBIAD1N102S (compare lane 8 with lane 9).

When transfected into UBIAD1-deficient cells, the amount of the T7-tagged membrane domain of wild type HMGCR (HMGCR (TM1-8)-T7) was low (Fig. 2e, lane 3). This is consistent with our previous observation that in both cultured cells and whole animals, ERAD of HMGCR was accelerated in the absence of UBIAD123,36. Co-expression of full-length, Myc-tagged UBIAD1N102S markedly stabilized HMGCR (Fig. 2e, lanes 5 and 6). HMGCRTM (V181R) and HMGCRTM (V267R) precipitated UBIAD1N102S, albeit at slightly reduced levels compared to HMGCRTM (lanes 4 and 7). We also generated a variant of UBIAD1N102S containing a point mutation (F172A) at the HMGCR-UBIAD1 complex interface (Fig. 2b, c). The results show that co-precipitation of UBIAD1N102S (F172A) with HMGCRTM was reduced compared to UBIAD1N102S (compare lane 8 with lane 9).

Structural analysis of UBIAD1N102S

Despite limited sequence similarities, the overall structure of hamster UBIAD1N102S resembles the previously reported structures of two archaeal UbiA prenyltransferases4,38 with a root-mean-square-deviation (RMSD) of 3.1 Å (Fig. 3a–c). Loops that separate the TM helices are relatively short except for the loop between TMs 2 and 3 (L2-3; amino acids 108–129) and the helix between TMs 6 and 7 (Hx6-7; amino acids 235–243). UbiA prenyltransferases contain two aspartate-rich motifs (NDXXDXXXD and DXXD) that are essential for enzymatic activity39.

These motifs, which correspond to N102TYYDFSKG and D236MESD in UBIAD1, are located at the C-terminal ends of TMs 2 and 6, respectively (Fig. 3a). Hx6-7, L2-3, and the loop between TMs 4 and 5 (L4-5) form a cap domain that lies over a central, negatively charged cavity generated by TMs 1, 2, 4, 5, and 6 (Fig. 3a). The structural analysis of the archaeal UbiA prenyltransferases suggests a model in which the cap domain adopts an open conformation in the absence of the isoprenyl substrate37,38. Binding of the substrate induces conformational changes in L2-3 that causes the cap domain to adopt a closed conformation that seals the enzyme’s active site (Fig. 3b). The structure of UBIAD1N102S reveals that the cap domain adopts an open conformation, which is consistent with previous findings that the N102S mutation reduces the affinity of UBIAD1 for GGpp20. Figure 3d shows the location of residues in UBIAD1 that are mutated in SCD. SCD-associated mutations cluster in L2-3 and Hx6-7 or line the central cavity that harbors the enzyme’s active site. These residues, many of which are conserved in UbiA prenyltransferases, are likely involved in binding of GGpp or catalysis. Indeed, our group and others have demonstrated that introduction of SCD-associated mutations in UBIAD1 reduces enzymatic activity20,33.

In comparing the structures of archaeal UbiA prenyltransferases (bound to substrate) and UBIAD1N102S, we noticed a significant difference. The central cavity in the archaeal enzymes has a lateral opening delineated by kinked TMs and TM94,9 (Fig. 3b, c). This lateral opening may allow these enzymes to accommodate longer isoprenyl substrates and/or release reaction products. Interestingly, TM1 of UBIAD1N102S forms an intact α-helix that blocks the lateral opening (Fig. 3a, c). It is tempting to speculate that binding to GGpp or MK-4 triggers conformational changes in TM1 that allows release of the product into the membrane bilayer.

Structural analysis of HMGCR

The overall structure of HMGCRTM revealed that TMs1–7 are integrated into membranes. The loops between TM1 and TM2 (25 amino acids in length) as well as TMs were not visualized in the structure, which indicates considerable flexibility within the regions (Fig. 1d). Further
analysis of the resolved structure revealed that HMGCR™ adopts two distinct conformations designated Conformation A and Conformation B (Fig. 4a, b). In UBIAD1-HMGCR complex 1 and 2b, HMGCR™ assumes Conformation A in which TM2 is perpendicular to the membrane, whereas TM4 is unwound to generate two half helices that we designate TM4a and TM4b (Fig. 4b). HMGCR™ assumes Conformation B in UBIAD1-HMGCR complex 2a. TM2 is tilted 45° in the membrane (Fig. 4a) and TM4 forms an intact a-helix (Fig. 4b). In the dimeric state of complex 2a, Conformation B becomes stabilized through direct interactions between TMs of HMGCR.

SSDs are found in five other proteins—Scap, Niemann-Pick C1 (NPC1), NPC1-Like1 (NPC1L1), Patched, and Dispatched—implicated in the regulation of cholesterol metabolism and signaling (Supplementary Fig. 10). Scap is a cholesterol-regulated escort protein required for activation of membrane-bound transcription factors called sterol regulatory element-binding proteins (SREBPs)\(^\text{40}\). NPC1 and NPC1L1 mediate intracellular transport of LDL-derived or dietary cholesterol\(^\text{16}\). Patched binds to the cholesterol-modified morphogen Hedgehog, while Dispatched mediates release of Hedgehog from cells\(^\text{2}\). Scap and HMGCR are unique among SSD-containing proteins in that sterols cause both proteins to bind Insig. However, Insig binding does not lead to accelerated ERAD of Scap. Instead, the reaction traps Scap in the ER, preventing its transport to the Golgi for proteolytic activation of bound SREBPs\(^\text{1}\).

The structures of NPC1, NPC1L1, Patched, Dispatched, and Scap in complex with Insig-2, have been determined\(^\text{44}\). TMs 2–6 constitute the SSD in HMGCR, Scap, and Patched, whereas TMs 3–7 constitutes the NPC1-SSD and NPC1L1-SSD. The TMs of NPC1, NPC1L1, Patched, and Dispatched contain at least 12 transmembrane helices including a pseudo-SSD that associates with the SSD to restrain its conformation \(\text{A} \rightarrow \text{B}\). These comparisons revealed the structure of HMGCR (TMs 1–6) in Conformation A is similar to that of Insig-bound Scap (Fig. 4c). TM2 in both SSDs is vertical in the membrane and remarkably, TM4 is broken at similar positions (Fig. 4c, Supplementary Fig. 10). The tilted conformation of TM2 when HMGCR adopts Conformation B interferes with the putative interaction between HMGCR and Insig. The clashes between Insig R110 and putative interaction between HMGCR and Insig. The clashes between Insig R110 and Insig complex. The TMs of Insig are indicated by underlining. Interactions between TMs of HMGCR.

Dynamic Reorganization of TMs in the HMGCR SSD

Further analysis of the two HMGCR SSD conformations may provide insight into mechanisms for the sterol-sensing reaction. Thus, we superimposed the structure of TMs 1–6 of HMGCR in Conformation A
with the previously reported structure of the Scap-Insig-2 complex. This superimposition indicates that the predicted HMGCR-Insig-2 interface is similar to the Scap-Insig interface, which is comprised of TM2, 4, and 5 of Scap and TM3 and 4 of Insig-2. A previous finding suggested that unwinding of Scap-TM4 exposes negatively charged E359, permitting interaction with R110 (and perhaps K102) of Insig-2 that stabilizes the Scap-Insig-2 complex. Our modeling predicts that D133 of HMGCR assumes a position equivalent to that of E359 in the broken TM4 of Scap and contributes to formation of the HMGCR-Insig-2 complex (Fig. 3b). The YIF motif of Scap and HMGCR, which is required for their sterol-induced binding to Insigs, is positioned similarly in TM2 of both proteins (Fig. 3b).

Figure 3c shows the superimposed structures of the Scap-Insig-2 complex and HMGCR (TM1-6) in Conformation B that results in rotation of TM2 and TM4 approximately 180° (Fig. 3a, c). This rotation causes significant steric hinderance between F80 and Insig-2-TM3; steric clash is also observed between I132 in HMGCR and R110 of Insig-2 (Fig. 3c). It is notable that in the absence of Insig-2, Scap-TM2 is not observed in the cryo-EM maps, which indicates considerable flexibility. TM4 is not broken and may adopt a continuous α-helix similar to that of HMGCR-TM4 in Conformation B (Supplementary Fig. 12).

Comparing the structure of HMGCR to that of Scap and other SSD-containing proteins led us to speculate that when HMGCR adopts Conformation B, the SSD cannot bind to Insigs and resists ERAD. However, adoption of Conformation A promotes binding of HMGCR to Insig for subsequent ubiquitination and ERAD. Analysis of the HMGCR structure led us to postulate that flexibility of the luminal loop between TM1 and TM2 (L1-2) may affect the conformation of TM2, leading to reorganization of TMs within the SSD that regulates HMGCR ERAD (Fig. 3e). Thus, we screened several variants of HMGCR harboring deletions within L1-2 that reduce flexibility and restrict the conformation of the SSD. We identified one variant, designated HMGCRΔ (Δ40–55), harboring a 16-mer acid deletion that exhibits reasonable expression yield and sufficient biochemical behavior. Cryo-EM analysis of the HMGCRΔ (Δ40–55)–UBIAD1 complex revealed that the L1-2 deletion caused HMGCR to exclusively assume Conformation B in which TM2 is tilted in the membrane; TM4 is intact regardless of its monomeric and dimeric state (Fig. 3f, Supplementary Figs. 3f, 13). To validate these structural observations, we examined the sterol-accelerated ERAD of T7-tagged HMGCR harboring the L1-2 deletion (designated HMGCR-T7 (Δ40–55)), HMGCR-T7 (WT) was subjected to Insig-mediated ERAD stimulated by the oxysterol 25-hydroxycholesterol (25-HC) and mevalonate (which provides a source of Gpp) (Fig. 5g, lanes 1–4), in contrast, HMGCR-T7 (Δ40–55) completely resisted 25-HC-induced ERAD (Fig. 5g, lanes 5–8). Co-immunoprecipitation was used to measure sterol-mediated association of HMGCR-T7 (WT) and (Δ40–55) with Insig-1. The results show that 25-HC enhanced the co-precipitation of Insig-1 with HMGCR-T7 (WT) (Fig. 5h, lanes 1-6), but not HMGCR-T7 (Δ40–55) (Fig. 5h, lanes 7-12). Based on these findings, we conclude that flexibility of L1-2 significantly contributes to reorganization of the SSD that permits binding of Insigs.

Discussion
Previous studies have described an intricate pathway through which distinct lipids—sterols and Gpp—accelerate Insig-mediated ERAD of HMGCR. A key breakthrough in the understanding of HMGCR ERAD came with the discovery that UBIAD1 binds to HMGCR and inhibits its ERAD. In the current studies, we analyze the structure of the HMGCR-UBIAD1 complex, which reveals a hydrophobic interface that is mediated by multiple interactions between the TMs of UBIAD1 and HMGCR (Fig. 1c–g). Mutation of key residues in this interface disrupts formation of the HMGCR-UBIAD1 complex and blunts UBIAD1-mediated stabilization of HMGCR (Fig. 2d–f). Identification of the HMGCR-UBIAD1 interface has important implications for the molecular basis of statin-induced accumulation of HMGCR that was described more than 40 years ago. Studies in genetically-manipulated mice revealed that inhibition of ERAD substantially contributes to statin-accumulation of HMGCR. UBIAD1 is sequestered in the ER of hepatic membranes isolated from statin-fed mice owing to depletion of Gpp and unconvincing genetic evidence has been observed that UBIAD1 is an inhibitor of HMGCR ERAD. Taken together with the current study, we predict molecules that disrupt the HMGCR-UBIAD1 interface or mimic Gpp in stimulating ER-to-Golgi translocation of UBIAD1 will enhance ERAD of HMGCR, preventing its accumulation associated with statin therapy.

We captured the SSD of HMGCR in two distinct conformations, indicating TMs in the region undergo dynamic reorganization within ER membranes (Fig. 5e). Importantly, the cytosolically-exposed sites for sterol-induced ubiquitination (K89 and K248) are identical in both conformations, ruling out the possibility that reorganization of the SSD alters access of the sites to ubiquitination machinery. We attenuated the structural rearrangement of TMs in the SSD through protein engineering and discovered that conformational transition to Conformation B (Fig. 5f) abolished its Insig-mediated ERAD stimulated by 25-HC (Fig. 5g). Certain oxysterols such as 24,25-dihydrolanosterol (DHL) accelerates Insig-mediated ERAD of HMGCR, but does not bind to Scap or Insig. We hypothesize that the SSD of HMGCR directly senses the concentration of DHL embedded in ER membranes, causing the protein to change its conformation to engage Insigs for ubiquitination and ERAD to control the synthesis of cholesterol. Although the structures of other SSD-containing proteins have been determined, these studies neither observed the dynamic reorganization of the SSD nor determined whether multiple conformations of the SSD modulated the protein’s activity. Our findings provide structural evidence validated by functional assays that are beginning to disclose the molecular basis through which SSDs control protein function.

Despite the advance in the understanding of the HMGCR ERAD pathway, several questions remain outstanding. For example, molecular mechanisms underlying transition of the HMGCR-SSD between Conformations A and B regulated by DHL and oxysterols remain to be determined. The precise mechanism through which UBIAD1 inhibits HMGCR ERAD at a post-ubiquitination step in the reaction has not been elucidated. Finally, mechanisms whereby Gpp dissociates the HMGCR-UBIAD1 complex and stimulates ER-to-Golgi transport of UBIAD1 is unknown. Attempts to address these important questions utilizing a variety of approaches are currently underway.

Methods
Expression plasmids
A cDNA encoding the transmembrane domain (amino acids 1–356) of hamster HMGCR harboring mutations (K89R, K248R) that abolish the enzyme’s sterol-induced ubiquitination was cloned into the pEZT-BM vector with a N-terminal FLAG tag. The resulting expression plasmid is designated pEZT-BM-FLAG-HMGCR. The cDNA for hamster UBIAD1 containing a 40-mer acid deletion at N-terminus and the SCD-associated N102S mutation was cloned into pEZT-BM vector preceded by a StrepII tag. BRIL insertion was screened through different replacement for loops between TMs of UBIAD1. The final construct used for cryo-EM structure determination is the one with BRIL insertion located between Phex and Pro309. This expression
plasmid is designated pE7Z-BM-StrepII-UBIAD1-BRILN102S. The following expression plasmids were described in the indicated reference: pCMV-HMGCR (TM1-S7) encoding amino acids 1-346 of hamster HMGCR with 3 copies of the T7 epitope at the C-terminus under transcriptional control of the cytomegalovirus (CMV) promoter; pCMV-Myc-UBIAD1 (N102S) encoding human UBIAD1 containing a Myc tag at the N-terminus under control of the CMV promoter; pCMV-Insig-I-Myc, which encodes human Insig followed by six copies of the Myc epitope under control of the CMV promoter; and pCMV-HMGCR-7, encoding full-length hamster HMGCR followed by three copies of the T7 epitope under control of the CMV promoter. Site-directed mutagenesis of pCMV-HMGCR (TM1-S7), pE7Z-BM-FLAG-HMGCR™, and pE7Z-BM-StrepII-UBIAD1N102S (without BRIL insertion) was carried out by two-step overlapping PCR. All mutations were verified by sequencing.

Cloning, expression, and purification of HMGCR™-UBIAD1N102S complex

pE7Z-BM-FLAG-HMGCR™ and pE7Z-BM-StrepII-UBIAD1-BRILN102S were introduced into HEK-293S GnTI cells (ATCC) by baculovirus-mediated transduction. Following incubation for 60 h at 30 °C, cells were harvested and disrupted by sonication in buffer A (20 mM HEPES pH 7.5, 150 mM NaCl) containing 1 mM PMSF, 10 μg/ml leupeptin. After low-speed centrifugation, the supernatant was incubated with 1% (w/v) lauryl maltose neopentyl glycol (LMNG, Anatrace), 0.1% cholesterol hemisuccinate (CHS, Steraloids) at 4 °C for 1 h. Lysates were clarified by centrifugation at 20,000 × g; the supernatant of this spin was loaded onto an anti-FLAG M2 affinity column (Sigma). After washing twice with buffer B (20 mM HEPES pH 7.5, 400 mM NaCl, 0.1% LMNG, 0.01% CHS, 10 μg/ml POPS, 10 μg/ml Soybean Polar Lipid Extract), bound proteins were eluted in buffer C (20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% LMNG, 0.01% CHS, 10 μg/ml POPS, 10 μg/ml Soybean Polar Lipid Extract and 0.1 mg/ml FLAG peptide). The sample was eluted with a Superose-6 column (GE Healthcare) in buffer A. Peak fractions containing the assembled complex were concentrated to ~10 mg/ml and 2 mM Fluorinated Fos-Choline-8 (Anatrace) was added to the sample before making grids. To assemble the HMGCR™-UBIAD1-BRILN102S complex, purified HMGCR™-UBIAD1-BRILN102S was first incubated with Amphipol AS-35 (Anatrace) for 4 h at 4 °C. The detergent was then removed by overnight incubation with Bio-beads (Bio-Rad). The amphipol-solubilized complex was mixed with Fab812 and Nb at 1:1:5:2.5 molar ratio for 1 hour at 4 °C. The HMGCR™-UBIAD1-BRILN102S-Fab812-Nb complex was finally purified with a Superose-6 column (GE Healthcare) in buffer A. Peak fractions containing the assembled complex were concentrated to ~10 mg/ml and 2 mM Fluorinated Fos-Choline-8 (Anatrace) was added to the sample before making grids. To assemble the HMGCR™-UBIAD1-BRILN102S-Fab812 complex, purified HMGCR™-UBIAD1-BRILN102S and Fab812 were mixed at 1:1:1 molar ratio and incubated on ice for 1 h, followed by gel-filtration with a Superose-6 column (GE Healthcare) in buffer D. Peak fractions that contained the HMGCR™-UBIAD1-BRILN102S-Fab812 complex were concentrated to ~10 mg/ml. Preparation of HMGCR™ (Δ40–55)-UBIAD1-BRILN102S-Fab812 complex sample was following the same procedure.

Cryo-EM imaging and data processing

The freshly purified complexes samples were added to Glow discharged Quantifoil R1.2/1.3 400 mesh Au holey carbon grids (Quantifoil), blotted using a Vitrobot Mark IV (FEI), and frozen in liquid ethane.

The grids were imaged in a 300 kV Titan Krios (FEI) with a Gatan K3 CCD detector. The specimen was imaged with a dose of 60 electrons per Å² using SerialEM57. Images were recorded for 5-second exposures in 50 subframes with a total dose of ~60 electrons per Å². Data were collected in super-resolution mode and the parameters of data collection are summarized in the Supplementary Table 1.

For all the three samples, Dark subtracted movie stacks were normalized by gain reference and the motion correction was performed using MotionCor2. The contrast transfer function (CTF) was estimated using CTFFIND4. After particle picking by cryoTOLE, the low-quality images and false-positive particles were removed manually. For the HMGCR™-UBIAD1-BRILN102S-Fab812-Nb complex, three data sets were collected. After 2D-classification of data set 1 in CryoSPARC®, classes with clean background were selected to generate initial models for the 3D-classification. Map from the best 3D class showing clear features of micelle, Fab and Nb were used as a model to fish out “good” particles from all the three data sets via 3D-classification. The resulting particles were subjected to the secondary 3D-classification with a mask in RELION-3. Particles from the good classes were polished and 3D classified in RELION-3. The best class was selected for the final 3D-refinement in RELION-3. For HMGCR™-UBIAD1-BRILN102S-Fab812 complexes, good classes from the initial 2D-classification were select to generated initial models for the 3D-classification of the entire particle set in CryoSPARC. The best 3D class was 2D classified and the classes showing features of monomer or

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The structure model was manually built by COOT and reconsrtucted from PDB: 6WW2 were docked into map as the initial model. The structure predicted by Swiss-Model were docked into the monomeric UBIAD1-(N102S (Δ40–55)), UBIAD1-BRII (N102S, Fab182) complexes, monomeric and dimeric maps from HMGCR (Δ40–55)-UBIAD1-BRII complexes were used as models for 3-classification in CryoSPARC. Particles from the monomeric and dimeric classes were applied to 2-classification and a secondary 3-classification to further exclude the bad particles. The remaining particles in the two classes were CTF refined by the final local 3D refinement in CryoSPARC, respectively.

**Model Construction, Refinement and Validation**

For the HMGCR (Δ40–55)-UBIAD1-BRII (N102S, Fab182) complex, a HMGCR (Δ40–55)-UBIAD1-BRII (N102S, Fab182) structure predicted by AlphaFold and a Fab (N102S, Fab182) structure predicted by SwissModel were docked into the Complex-1 map as the initial model. The refined Complex-1 structure was docked into the Complex-1 region of the Complex-1 map used as the initial model for Complex-2a. Both structure models were manually built by COOT followed by refinement in real space using PHENIX and reciprocal space using Refmac with secondary-structure restraints and stereochemical restraints. For cross-validation, the final model was refined with the half map 1 from the final 3D-refinement. The resulting model was used to calculate the model vs. map FSC curve against half map 1 and 2, respectively, using the Comprehensive validation module in PHENIX. MolProbity was used to validate the geometries of the structure. Structure figures were generated using PyMOL (http://www.pymol.org), Chimera and ChimeraX. The Complex-1 structure with the deletion of TMs 7–8 of UBIAD1 was docked into the Complex-2b region of the Complex-2 map as the initial model for Complex-2b. The Complex-2b structure was refined once in real space using PHENIX for figures. For the HMGCR (Δ40–55)-UBIAD1-BRII (N102S, Fab182)-Nb complex, HMGCR (Δ40–55)-UBIAD1-BRII (N102S, Fab182)-N2 complex from PDB: 6WW2 were docked into map as the initial model. The structure model was manually built by COOT and refined once in real space using PHENIX for figure preparation. For HMGCR (Δ40–55)-UBIAD1-BRII (N102S, Fab182) complexes, structure of HMGCR (Δ40–55)-UBIAD1-BRII (N102S, Fab182) Complex-2a was docked into the monomeric and dimeric maps, respectively, as initial models. The structure models were manually built by COOT and refined once in real space using PHENIX for figure preparation.

**Transient Transfection and Immunoprecipitation**

HEK-293S GnTI cells were maintained in suspension in FreeStyle 293 expression medium (Gibco, Cat# 12338-026) containing 2% FCS, 100 units/ml penicillin and 100 mg/ml streptomycin sulfate at 37 °C, 8% CO2. Cells were set up for experiments on day 0 at the density of 0.6 × 106 cells per 60-mm dish. On day 1, cells were transfected with variants of pCMV-HMGCR (Δ40–55) and pE31-AM-UBIAD1 using X-tremeGENE HP transfection reagent (Roche) (3 μl/μg DNA). The cells were depleted of sterol and nonsterol isoprenoids through incubation in medium supplemented with lipoprotein-deficient serum, 10 μM of the statin compactin, and 50 μM sodium mevalone. After 24 h at 37 °C, cells were harvested from transfected UT-2 cells (Fig. 5g, h) were maintained as described previously and set up for experiments on day 0 at 5 × 105 cells per 60-mm dish. On day 1, cells were transfected with pCMV-HMGCR-T7 (WT) or (Δ40–55) (1 μg/dish) in the absence or presence of 10–30 ng of pCMV-Insig-1-Myc using X-tremeGENETM-360 transfection reagent (Roche) (3 μl/μg DNA). The cells were depleted of sterol and nonsterol isoprenoids through incubation in medium supplemented with lipoprotein-deficient serum, 10 μM of the statin compactin, and 50 μM sodium mevalone.

**Statistics and reproducibility**

The experiments in Figs. 2d–f, 5g, h and Supplementary Figs. 2, 3a–c were repeated at least two times on different days. Similar results were obtained.

**Reporting summary**

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Data supporting the findings of this manuscript are available from the corresponding author upon reasonable request. The 3D cryo-EM density maps have been deposited in the Electron Microscopy Data Bank under the accession numbers EMD-27461 (complex in state 1), EMD-27460 (complex in state 2), EMDB-27475 (complex in amphipols), EMDB-27478 (Complex Δ40–55 in state 1), and EMDB-27477 (Complex Δ40–55 in state 2). Atomic coordinates for the models have been deposited in the Protein Data Bank under the accession numbers 6DJM [https://doi.org/10.2210/pdb6DJM/pdb] (complex in state 1) and 8DJK [https://doi.org/10.2210/pdb8DJK/pdb] (complex in state 2). Source Data underlying Figs. 2d–f and 5g, h are provided as a Source Data file. Source data are provided with this paper.

**References**

1. Goldstein, J. L. & Brown, M. S. Regulation of the mevalonate pathway. Nature 343, 425–430 (1990).
2. Brown, M. S. & Goldstein, J. L. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J. Lipid Res.* **21**, 505–517 (1980).

3. Schumacher, M. M. & DeBoe-Boyd, R. A. Post-translational regulation of HMG CoA reductase, the rate-limiting enzyme in synthesis of cholesterol. *Annu. Rev. Biochem.* **90**, 659–679 (2021).

4. Luo, J., Yang, H. & Song, B. L. Mechanisms and regulation of cholesterol homeostasis. *Nature Rev. Molecular Cell Biol.* [https://doi.org/10.1038/s41580-019-0190-7] (2019).

5. Sharpe, L. J., Coates, H. W. & Brown, A. J. Post-translational control of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Biol. Chem.* **295**, 17549–17559 (2020).

6. Nakashima, M., Goldstein, J. L. & Brown, M. S. Multivalent control of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Nature*. [amembrane -](10.1038/s41580-019-0190-7)

7. Ravid, T., Doolman, R., Avner, R., Harats, D. & Roitelman, J. The prenyltransferase UBIAD1 is the target of geranylgeranylregulated transport of the prenyltransferase UBIAD1 between membranes of the ER and Golgi. *J. Lipid Res.* **57**, 1286–1299 (2016).

8. Nakanishi, M., Goldstein, J. L. & Brown, M. S. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J. Lipid Res.* **21**, 505–517 (1980).

9. Schumacher, M. M., Jun, D. J., Johnson, B. M. & DeBoe-Boyd, R. A. UbiA prenyltransferase domain-containing protein-1 modulates HMG-CoA reductase degradation to coordinate synthesis of sterol and nonsterol isoprenoids. *J. Biol. Chem.* **293**, 312–323 (2018).

10. Schumacher, M. M., Jun, D. J., Y., Seemann, J. & DeBoe-Boyd, R. A. Geranylgeranyl-regulated transport of the prenyltransferase UBIAD1 between membranes of the ER and Golgi. *J. Lipid Res.* **57**, 1286–1299 (2016).

11. Orr, A. et al. Mutations in the UBIAD1 gene, encoding a potential prenyltransferase, are causal for a novel X-linked corneal dystrophy. *PLoS ONE* **2**, e6885 (2007).

12. Weiss, J. S. et al. Mutations in the UBIAD1 gene on chromosome 25q13-25q14 cause corneal dystrophy. *J. Clin. Investig.* **134**, 1905–1910 (2015).

13. Jo, Y. et al. Enhanced ER-associated degradation of HMG CoA reductase mediated by binding of Insig-1 to its sterol-sensing domain. *Mol. Cell** 8**, 392–393 (1988).

14. Schumacher, M. M. & DeBose-Boyd, R. A. Posttranslational regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Nature Rev. Mol. Cell Biol.* [https://doi.org/10.1038/s41580-019-0190-7](10.1038/s41580-019-0190-7)

15. Brown, M. S. & Goldstein, J. L. Multivalent feedback regulation of HMG-CoA reductase, the rate-limiting enzyme in synthesis of cholesterol. *J. Biol. Chem.* **275**, 35840–35847 (2000).

16. Sever, N., Yang, T., Brown, M. S., Goldstein, J. L. & DeBoe-Boyd, R. A. Accelerated degradation of HMG CoA reductase mediated by binding of Insig-1 to its sterol-sensing domain. *Mol. Cell** 11**, 25–33 (2003).

17. Ravid, T., Doolman, R., Avner, R., Harats, D. & Roitelman, J. The ubiquitin-proteasome pathway mediates the regulated degradation of mammalian 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem.* **275**, 52479–52490 (2003).

18. Skalnik, D. G., Narita, H., Kent, C. & Simoni, R. D. The membrane domain of 3-hydroxy-3-methylglutaryl-coenzyme A reductase confers endoplasmic reticulum localization and sterol-regulated degradation onto beta-galactosidase. *J. Biol. Chem.* **268**, 6836–6841 (1993).

19. Roitelman, J., Olender, E. H., Bar-Nun, S., Dunn, W. A. J. & Simoni, R. D. Immunological evidence for eight spans in the membrane domain of 3-hydroxy-3-methylglutaryl coenzyme A reductase: implications for enzyme degradation in the endoplasmic reticulum. *J. Cell Biol.* **117**, 599–597 (1992).

20. Jo, Y., Lee, P. C., Suguina, P. V. & DeBoe-Boyd, R. A. Sterol-induced degradation of HMG CoA reductase depends on interference of two Insigs and two ubiquitin ligases, gp78 and Trc8. *Proc. Natl. Acad. Sci. USA* **108**, 20503–20508 (2011).

21. Song, B. L., Sever, N. & DeBoe-Boyd, R. A. Gp78, a membrane-anchored ubiquitin ligase, associates with Insig-1 and couples sterol-regulated ubiquitination to degradation of HMG CoA reductase. *Mol. Cell** 19**, 829–840 (2005).

22. Jiang, L. Y. et al. Ring finger protein 145 (RNF145) is a ubiquitin ligase for sterol-induced degradation of HMG-CoA reductase. *J. Biol. Chem.* **293**, 4047–4055 (2018).

23. Morris, L. L., Hartman, I. Z., Jun, D. J., Seemann, J. & DeBoe-Boyd, R. A. Sequential actions of the AAA-ATPase Valosin-containing Protein (VCP)/p97 and the Proteasome 19 S regulatory particle in sterol-accelerated, endoplasmic reticulum (ER)-associated degradation of 3-Hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem.* **289**, 19053–19066 (2014).

24. Hwang, S. et al. Contribution of accelerated degradation to feedback regulation of 3-Hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol metabolism in the liver. *J. Biol. Chem.* **291**, 13479–13494 (2016).

25. Schumacher, M. M., Elsabrouy, R., Seemann, J., Jo, Y. & DeBoe-Boyd, R. A. The prenyltransferase UBIAD1 is the target of geranylgeranyl in degradation of HMG CoA reductase. *eLife* **4**, [https://doi.org/10.7554/eLife.05560](https://doi.org/10.7554/eLife.05560) (2015).
39. Li, W. Bringing bioactive compounds into membranes: the UbiA superfamily of intramembrane aromatic prenyltransferases. Trends Biochem. Sci. 41, 356–370 (2016).

40. Brown, M. S., Radhakrishnan, A. & Goldstein, J. L. Retrospective on cholesterol homeostasis: the central role of Scap. Annu. Rev. Biochem. 87, 783–807 (2018).

41. Pfeffer, S. R. NPC intracellular cholesterol transporter 1 (NPC1)-mediated cholesterol export from lysosomes. J. Biol. Chem. 294, 1706–1709 (2019).

42. Qi, X. & Li, X. Mechanistic insights into the generation and transduction of Hedgehog signaling. Trends Biochem. Sci. 45, 397–410 (2020).

43. Yang, T. et al. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. Cell 110, 489–500 (2002).

44. Li, X. et al. Structure of human Niemann-Pick C1 protein. Nature 504, 1314 (2013).

45. Schmiegel, P., Coutavas, E., Wang, J. & Li, X. Structures of human Patched and its complex with native palmitoylated sonic hedgehog. Nature 560, 128–132 (2018).

46. Qi, X., Schmiegel, P., Coutavas, E. & Li, X. Two Patched molecules engage distinct sites on Hedgehog yielding a signaling-competent complex. Science, https://doi.org/10.1126/science.aas8843 (2018).

47. Zhang, Y. et al. Structure of human Niemann-Pick C1 protein. Proc. Natl Acad. Sci. USA 113, 8212–8217 (2016).

48. Cannac, F. et al. Cryo-EM structure of the Hedgehog release protein dispatched. Sci. Adv. 6, eay7928 (2020).

49. Kober, D. L. et al. Scap structures highlight key role for rotation of intertwined luminal loops in cholesterol sensing. Cell 184, 3689–3701 e3622 (2021).

50. Yan, R. et al. A structure of human Scap bound to Insig-2 suggests how their interaction is regulated by sterols. Science 371, https://doi.org/10.1126/science.abb2224 (2021).

51. Long, T. et al. Structural basis for cholesterol transport-like activity of the hedgehog receptor patched. Cell 175, 1352–1364 e1314 (2018).

52. Cannac, F. et al. Cryo-EM structure of the Hedgehog release protein dispatched. Sci. Adv. 6, eay7928 (2020).

53. Wang, Q. et al. Dispatched uses Na(+) flux to power release of lipid-modified Hedgehog. Nature 599, 320–324 (2021).

54. Morales-Perez, C. L., Noviello, C. M. & Hibbs, R. E. Manipulation of subunit stoichiometry in heteromeric membrane proteins. Structure 24, 797–805 (2016).

55. Tsutsumi, N. et al. Structure of human Frizzled5 by fiducial-assisted cryo-EM supports a heterodimeric mechanism of canonical Wnt signaling. eLife 9, e58464 (2020).

56. Wang, Q. et al. A combination of human broadly neutralizing antibodies against Hepatitis B virus HBsAg with distinct epitopes suppresses escape mutations. Cell Host Microbe 28, 335–349 e336 (2020).

57. Mastronarde, D. N. Automated electron microscope tomography using robust prediction of specimen movements. J. Struct. Biol. 152, 36–51 (2005).

58. Zheng, S. Q. et al. MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. Nat. Methods 14, 331–332 (2017).

59. Rohou, A. & Grigorieff, N. CTFFIND4: Fast and accurate defocus estimation from electron micrographs. J. Struct. Biol. 192, 216–221 (2015).

60. Wagner, T. et al. SPHIRE-crYOLO is a fast and accurate fully automated particle picker for cryo-EM. Commun. Biol. 2, 218 (2019).

61. Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoS-PARC: algorithms for rapid unsupervised cryo-EM structure determination. Nat. Methods 14, 290–296 (2017).

62. Zivanov, J. et al. New tools for automated high-resolution cryo-EM structure determination in RELION-3. eLife 7, https://doi.org/10.7554/eLife.42166 (2018).

63. Jumper, J. et al. Highly accurate protein structure prediction with AlphaFold. Nature 596, 583–589 (2021).

64. Waterhouse, A. et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic acids Res. 46, W296–W303 (2018).

65. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. Acta Crystallogr. Sect. D. Biol. Crystallogr. 60, 2126–2132 (2004).

66. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. Sect. D. Biol. Crystallogr. 66, 213–221 (2010).

67. Murshudov, G. N. et al. REFMACS for the refinement of macromolecular crystal structures. Acta Crystallogr. Sect. D. Biol. Crystallogr. 67, 355–367 (2011).

68. Shen, V. B. et al. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr. Sect. D. Biol. Crystallogr. 66, 12–21 (2010).

69. Pettersen, E. F. et al. UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1621 (2004).

70. Pettersen, E. F. et al. UCSF ChimeraX: Structure visualization for researchers, educators, and developers. Protein Sci. 30, 70–82 (2021).

71. Mosley, S. T., Brown, M. S., Anderson, R. G. & Goldstein, J. L. Mutant clone of Chinese hamster ovary cells lacking 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Biol. Chem. 258, 13875–13881 (1983).

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Author contributions

R.D.B. and X.L. conceived the project and designed the research with H.C., X.Q., H.C. and X.Q. performed biochemical studies and carried out cryo-EM experimentation. H.C., X.Q., and L.D. screened the antibody. H.C., R.A.F., M.M.S. performed functional characterization experiments. X.Q. built the initial models and refined the structures. All of the authors analyzed the data and contributed to manuscript preparation. R.D.B. and X.L. wrote the manuscript.

Competing interests

The authors declare no competing interests.
