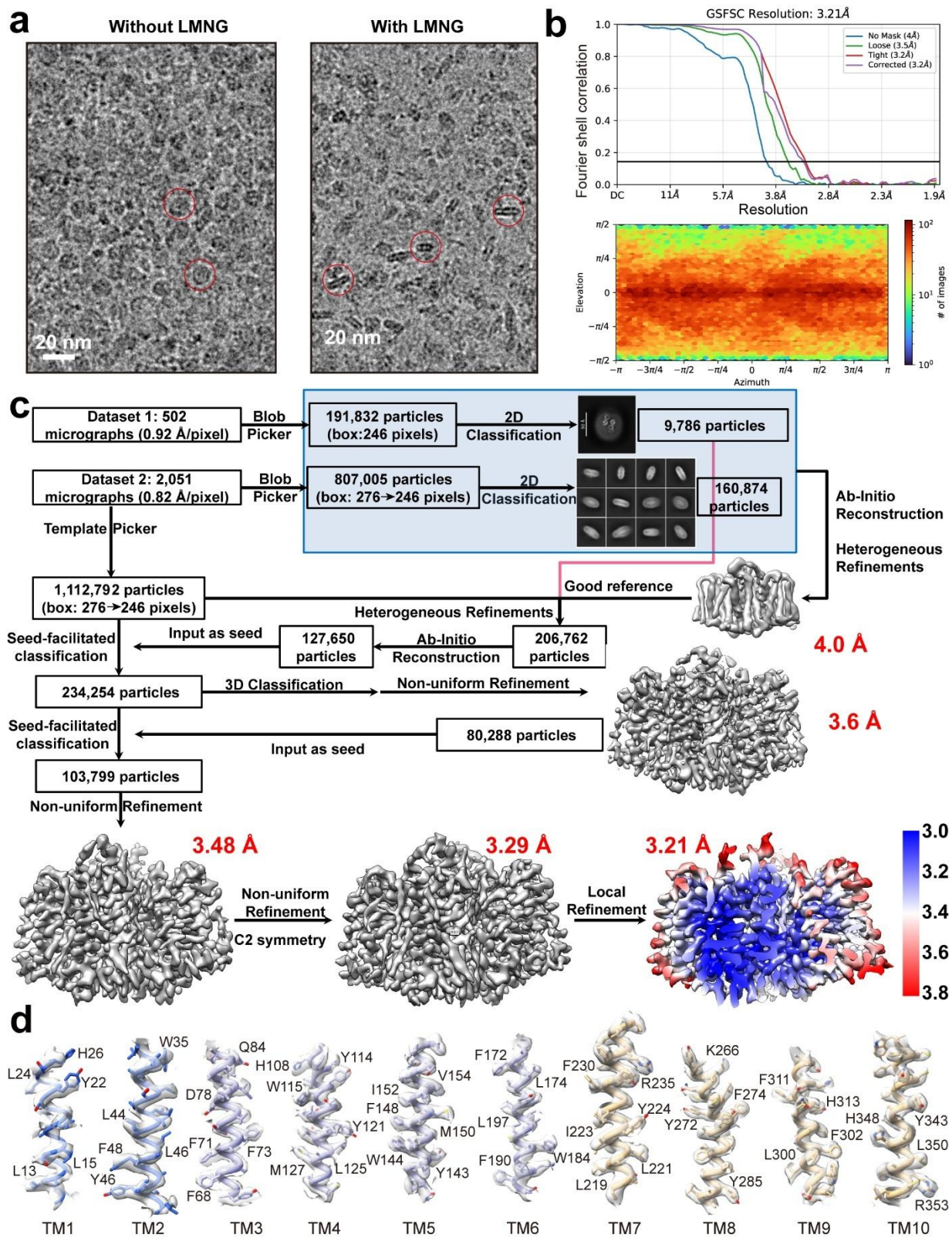
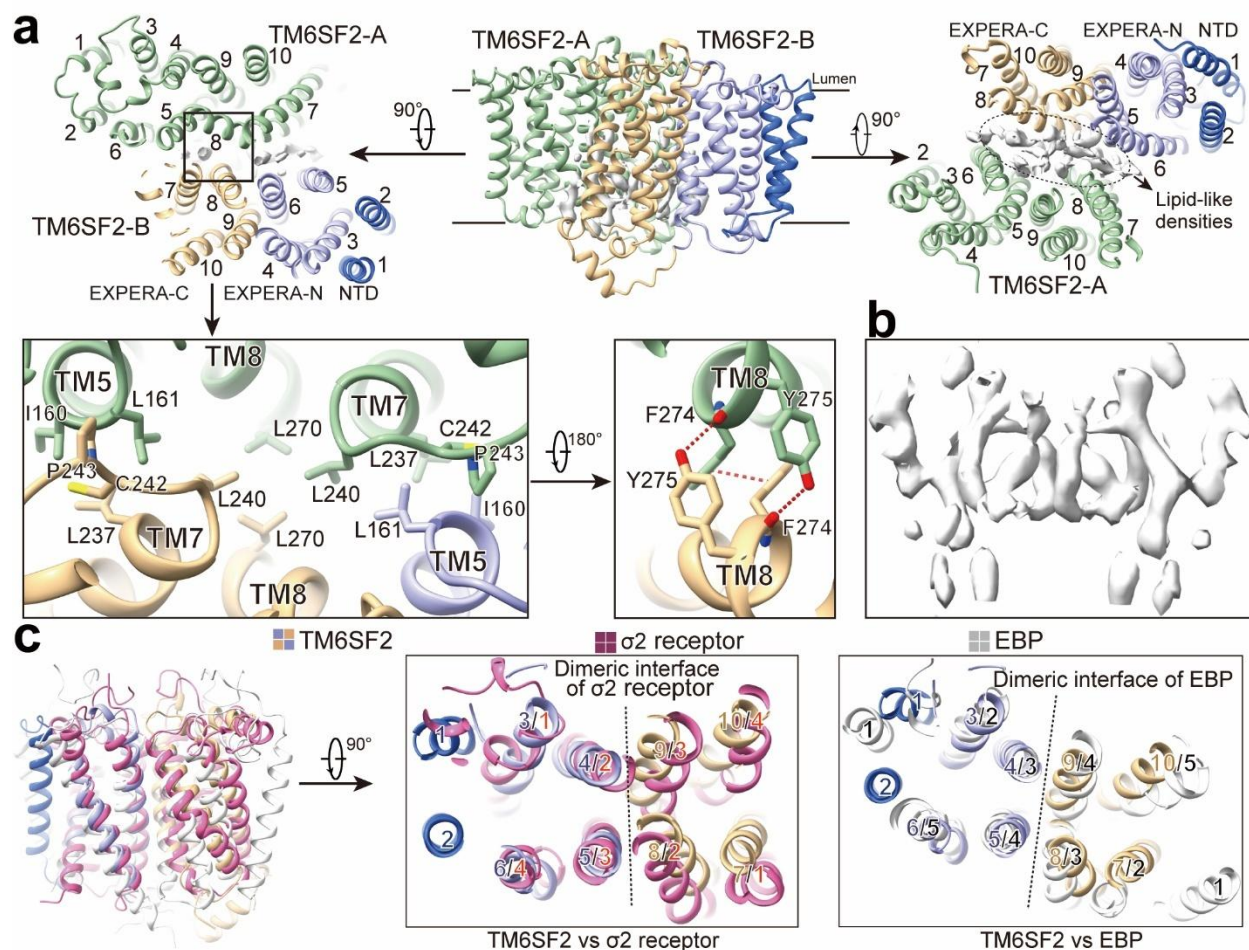




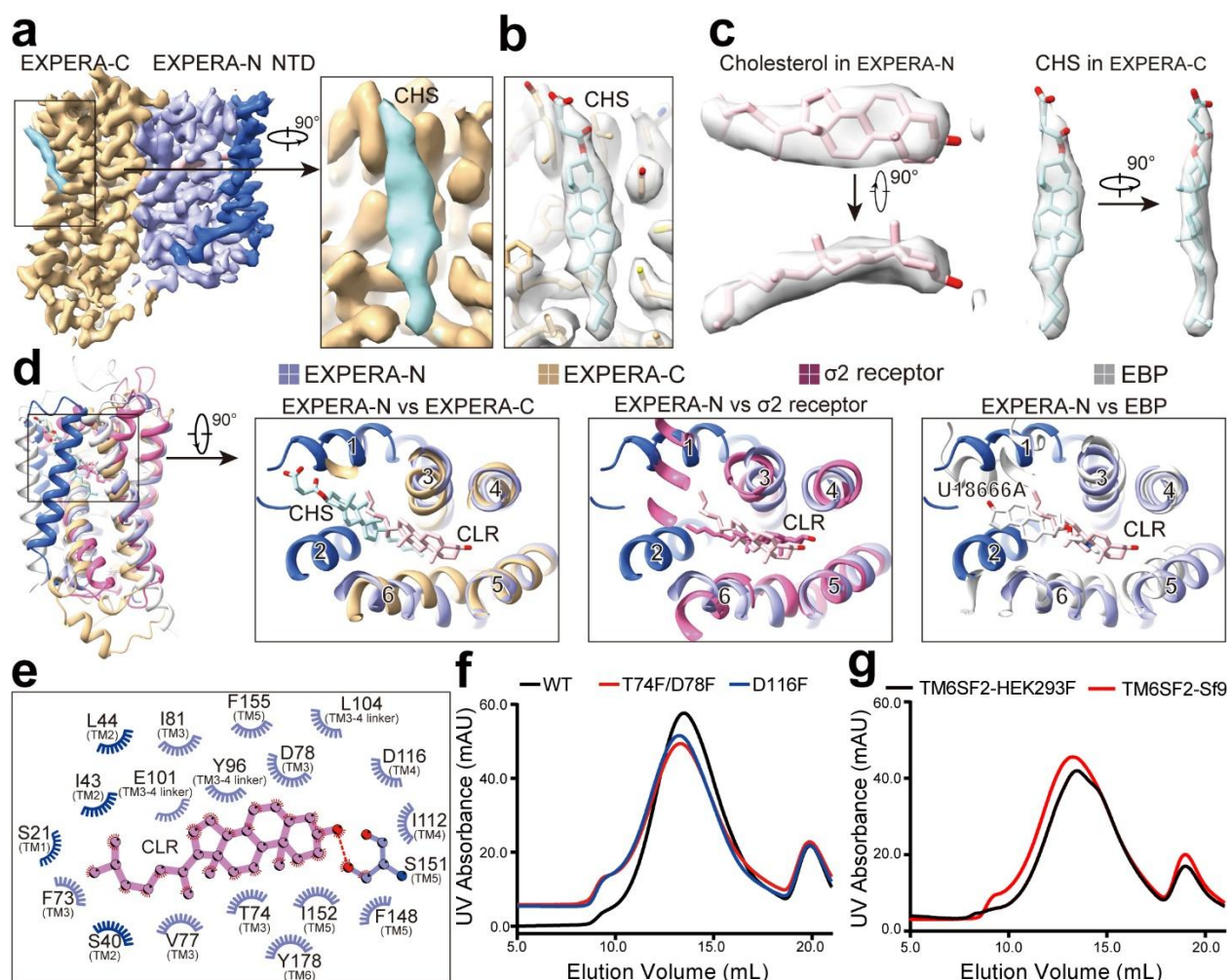
Extended Data Fig. 1| Structural determination of human TM6SF2 (hTM6SF2). a, Representative cryo-EM micrographs of hTM6SF2 nanodiscs in the absence (*left*) and presence

(*right*) of LMNG. The particles are indicated with red circles. **b**, Gold standard Fourier shell correlation (GSFSC) curves (*top*) and angular distribution map (*bottom*) for the final reconstruction. **c**, Flowchart of data processing. See Methods for details. The local resolution map for the final reconstruction was calculated using cryoSPARC¹ and generated with Chimera². The unit for resolution is Å. **d**, EM maps for representative elements of hTM6SF2.



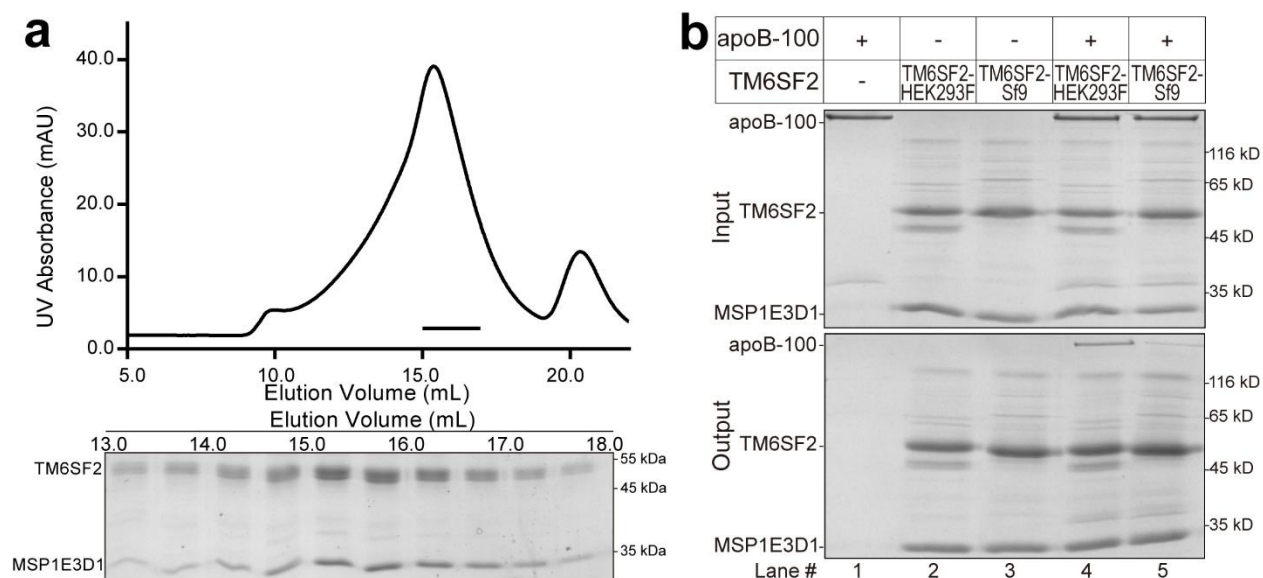
Extended Data Fig. 2| Dimeric organization of hTM6SF2. **a**, Dimeric interface between two protomers in the hTM6SF2 structure. *Top*: luminal (*left*), membrane (*middle*) and cytosolic (*right*) views of the dimeric structure. *Bottom*: two zoomed-in views are generated to show the hydrophobic interface on the luminal side (*left*) and the polar interactions in the mid-transmembrane region (*right*). The TM6SF2-A protomer is colored green, while the TM6SF2-B protomer is domain-colored according to the color code in Fig. 1d. Hydrogen-bond interactions and π - π interactions are indicated with dashed red lines. **b**, Lipid-like densities sandwiched between the two protomers in the hTM6SF2 structure. The densities are contoured at 5σ . **c**, Structural overlay of the hTM6SF2 protomer with the dimeric structures of EBP (PDB: 6OHT) and the $\sigma 2$ receptor (PDB: 7MFI). *Left*: membrane view showing the superimposition of the three structures relative to the EXPERA-N domain. *Middle and right*: two luminal views are generated

to show the structural overlay of the hTM6SF2 protomer with the σ 2 receptor (*middle*) and EBP (*right*), respectively.

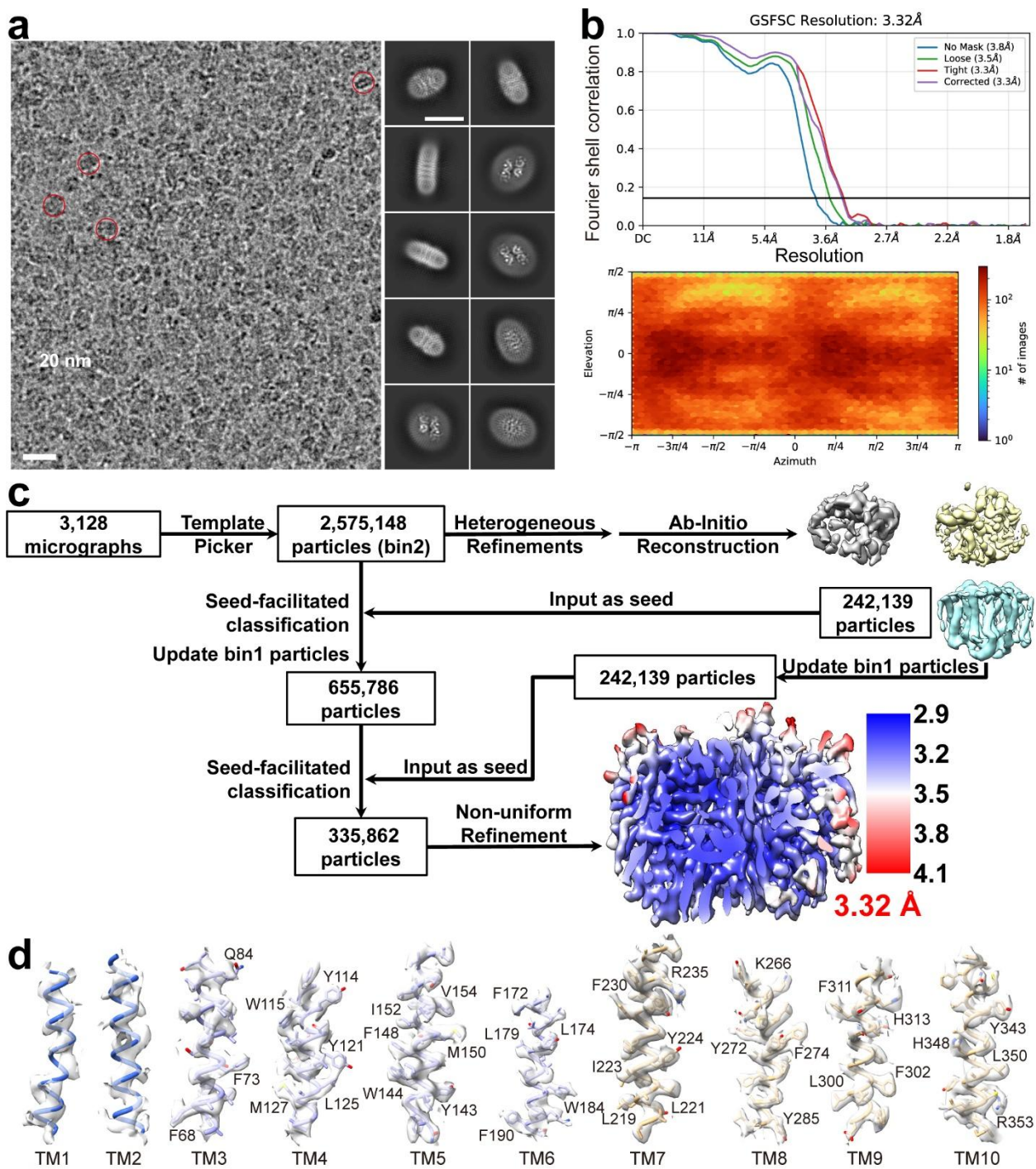


Extended Data Fig. 3 | Sterol-like densities in the EXPERA domains of hTM6SF2. **a**, Extra sterol-like densities are observed in a membrane-facing cavity of the EXPERA-C domain. *Inset*: A zoomed-in view to show the sterol-like densities highlighted in cyan. **b**, Modeling of a CHS molecule into the sterol-like densities in the EXPERA-C domain. **c**, Two perpendicular views are generated to show the modeling of cholesterol (*left*) and CHS molecules (*right*) into the sterol-like densities in the EXPERA-N and EXPERA-C domains, respectively. The densities for cholesterol and CHS are contoured at 5σ and 6σ , respectively. **d**, Structural superimposition of the EXPERA-N and EXPERA-C domains with the $\sigma 2$ receptor (PDB: 7MFI) and EBP (PDB: 6OHT). *Left*: A membrane view of the overlay for all these structures. *Right*: three zoomed-in views showing the pairwise comparisons between EXPERA-N with EXPERA-C, the $\sigma 2$ receptor, and EBP. The $\sigma 2$

receptor and EBP structures are colored red and white, respectively. **e**, Schematic elucidation of the coordination of cholesterol by the NTD and EXPERA-N domains. This figure was prepared with LigPlot⁺³. CLR, cholesterol; TM3-4 linker, the luminal loop connecting TM3 and TM4. **f**, SEC profiles of TM6SF2 variants (WT, T74F/D78F, and D116F) purified from HEK293F cells. **g**, Comparison of the SEC profiles of TM6SF2 purified from HEK293F (TM6SF2-HEK293F) and Sf9 (TM6SF2-Sf9) cells.

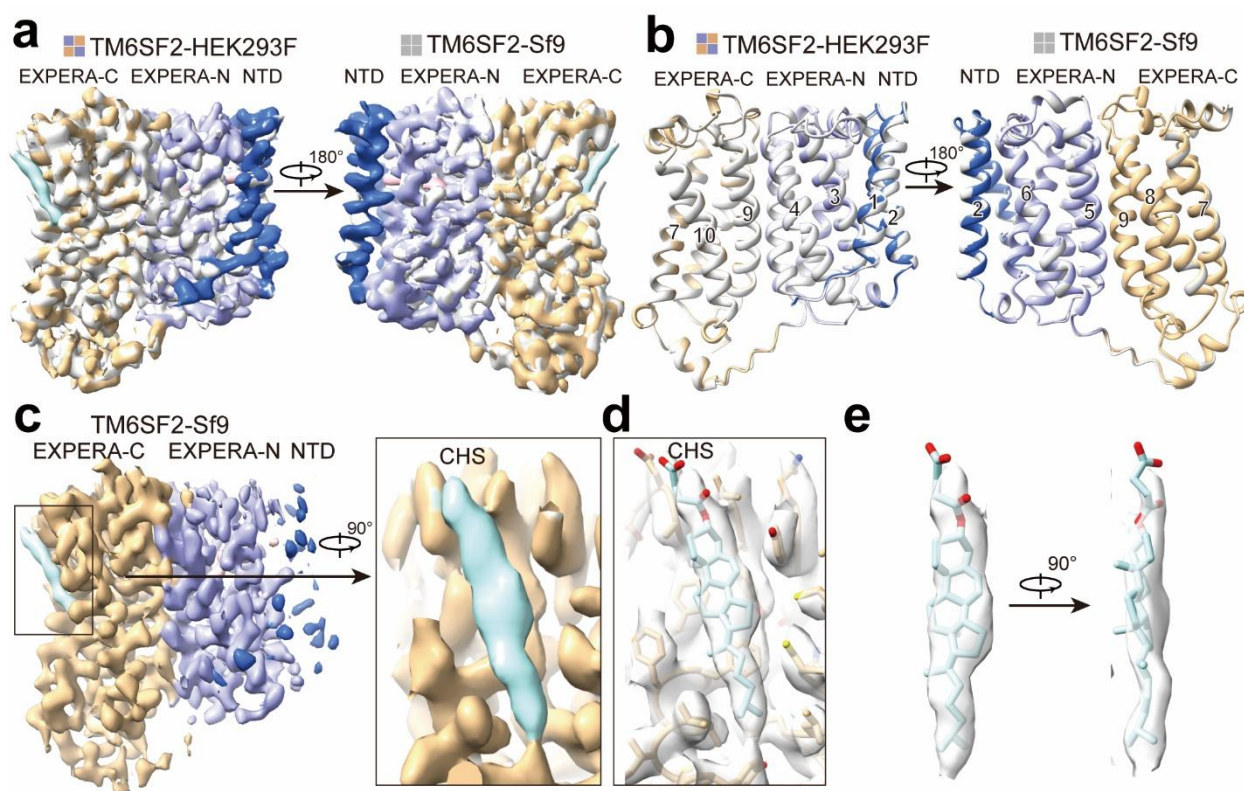


Extended Data Fig. 4| TM6SF2–apoB-100 interaction under nanodisc conditions. a, SEC profile of Sf9-expressing TM6SF2 reconstituted into the nanodiscs with MSP1E3D1 as the scaffold protein. After SDS-PAGE analysis, the peak fractions (indicated by black line) were concentrated for cryo-EM sample preparation. **b**, Evaluation of the interaction between apoB-100 and TM6SF2 nanodiscs. TM6SF2 nanodiscs were reconstituted using proteins expressed in HEK293F (TM6SF2-HEK293F) and Sf9 (TM6SF2-Sf9) cells.

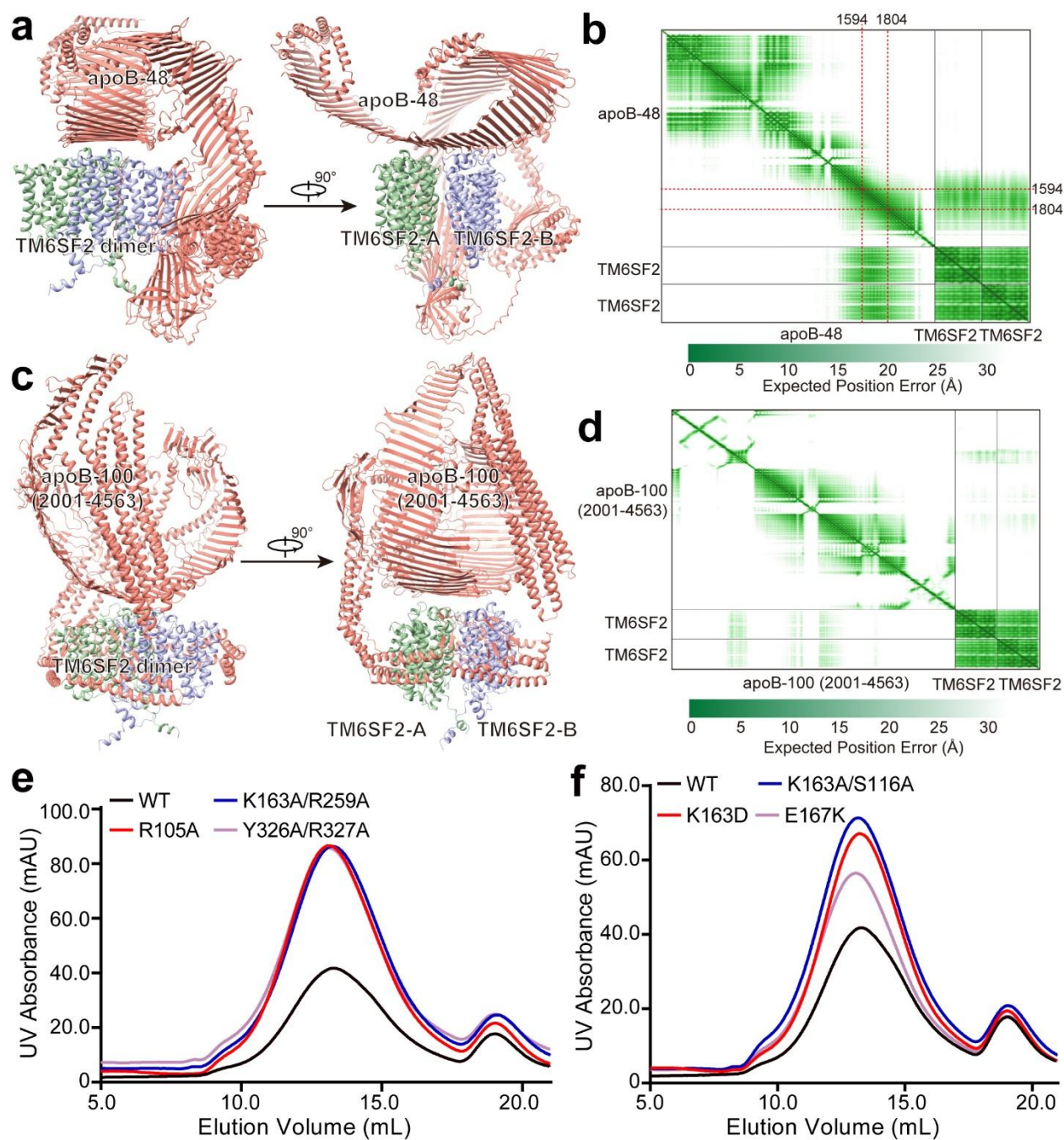


Extended Data Fig. 5| Cryo-EM structure determination of TM6SF2-Sf9. **a**, Representative micrograph (*left*) and 2D averages (*right*) for the cryo-EM sample of TM6SF2-Sf9. **b**, Gold standard Fourier shell correlation (GSFSC) curves (*top*) and angular distribution map (*bottom*) for

the final reconstruction. **c**, Flowchart of data processing. See Methods for details. **d**, EM maps for representative elements of TM6SF2-Sf9.

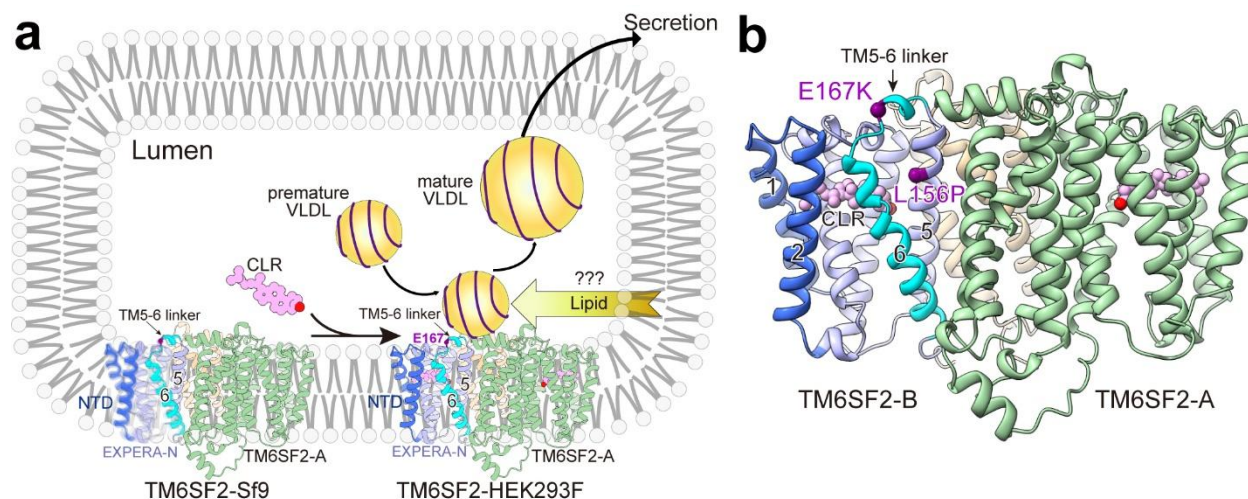


Extended Data Fig. 6 | Sterol-like densities in the EXPERA-C domains of TM6SF2-Sf9. **a**, Two perpendicular views are generated to show the map overlay of TM6SF6-HEK293F and TM6SF6-Sf9. **b**, Structural superimposition of TM6SF6-HEK293F and TM6SF6-Sf9. Only one protomer is shown in panels **a** and **b**. **c**, Extra sterol-like densities are observed in the membrane-facing cavity of the EXPERA-C domain. *Inset*: A zoomed-in view highlighting the sterol-like densities. **d**, Modeling of a CHS molecule into the sterol-like densities. **e**, Two perpendicular views are generated to show that the CHS molecule is well fitted with sterol-like densities in the EXPERA-C domain. The densities are contoured at 6σ .



Extended Data Fig. 7 | AlphaFold3-predicted structure of the complex between the TM6SF2 dimer and apoB proteins. **a**, Two perpendicular views are generated to show the predicted structure of the TM6SF2 dimer in complex with apoB-48, a shorter isoform comprising the N-terminal region (residues 1-2179) of apoB-100. **b**, Expected position error plot for the predicted structure in panel **a**. The region comprising residues 1594-1804 in apoB-48, which shows a high-

confidence interaction prediction, is highlighted with dotted red lines. **c**, Two orthogonal views are generated to show the predicted structure of the TM6SF2 dimer in complex with the C-terminal region of apoB-100 (residues 2001-4563). **d**, Expected position error plot for the predicted structure in panel **c**. **e, f**, SEC profiles of TM6SF2 variants harboring designed mutations that disrupt the predicted interfaces. The same WT profile is shown in panels **e** and **f**.



Extended Data Fig. 8| A proposed model for the function of TM6SF2 in VLDL lipidation. a,

Proposed model for bulk lipidation of premature VLDL in the ER lumen. In this model, cholesterol maintains a stable conformation of the NTD and EXPERA-N domains, thereby promoting a more stable conformation of the TM5-6 linker, which is essential for the interaction between TM6SF2 and premature VLDL. After stable interactions are established, lipids are transported from the ER membrane to premature VLDL via an unelucidated mechanism to promote its maturation. Finally, mature VLDL is released via the secretory pathway. **b,** Mapping of disease mutations onto the TM6SF2 structure. Two disease mutations, E167K and L156P, are shown as purple spheres and labeled in purple.

References

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- 2 Pettersen, E. F. *et al.* UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* **25**, 1605-1612, doi:10.1002/jcc.20084 (2004).
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