Expanded View Figures

Figure EV1. Sequence alignments of yeast Lac1 and Lag1, and human CerS family members.

Secondary structural elements of yeast Lac1 are labeled above the sequence alignment. CH: cytosolic helix; TM: transmembrane helix; LH: luminal helix. The NTD is colored wheat. The TLC domain is colored marine with the Lag1p motif shown in yellow. The four red triangles indicate the predicted catalytic histidine and aspartate residues in the Lag1p motif. The Hox domains and acyl-CoA selective regions in human CerS are highlighted with wheat and dark pink boxes, respectively. The DxRSDxE dimerization motif is highlighted with a green box. The UniProt IDs for the aligned sequences are as follows: yLac1: P28496; yLag1: P38703; hCerS1: P27544; hCerS2: Q96G23; hCerS3: Q8IU89; hCerS4: Q9HA82; hCerS5: Q8N5B7; and hCerS6: Q6ZMG9. "y" for Saccharomyces cerevisiae (yeast) and "h" for Homo sapiens (human).


CH1
TM1
LH1
TM2
TM3
Hox domain
LH2
TM4
TM5
TM6
TM7
TM8
Acyl-CoA selective region
DxRSDxE motif

Figure EV1.

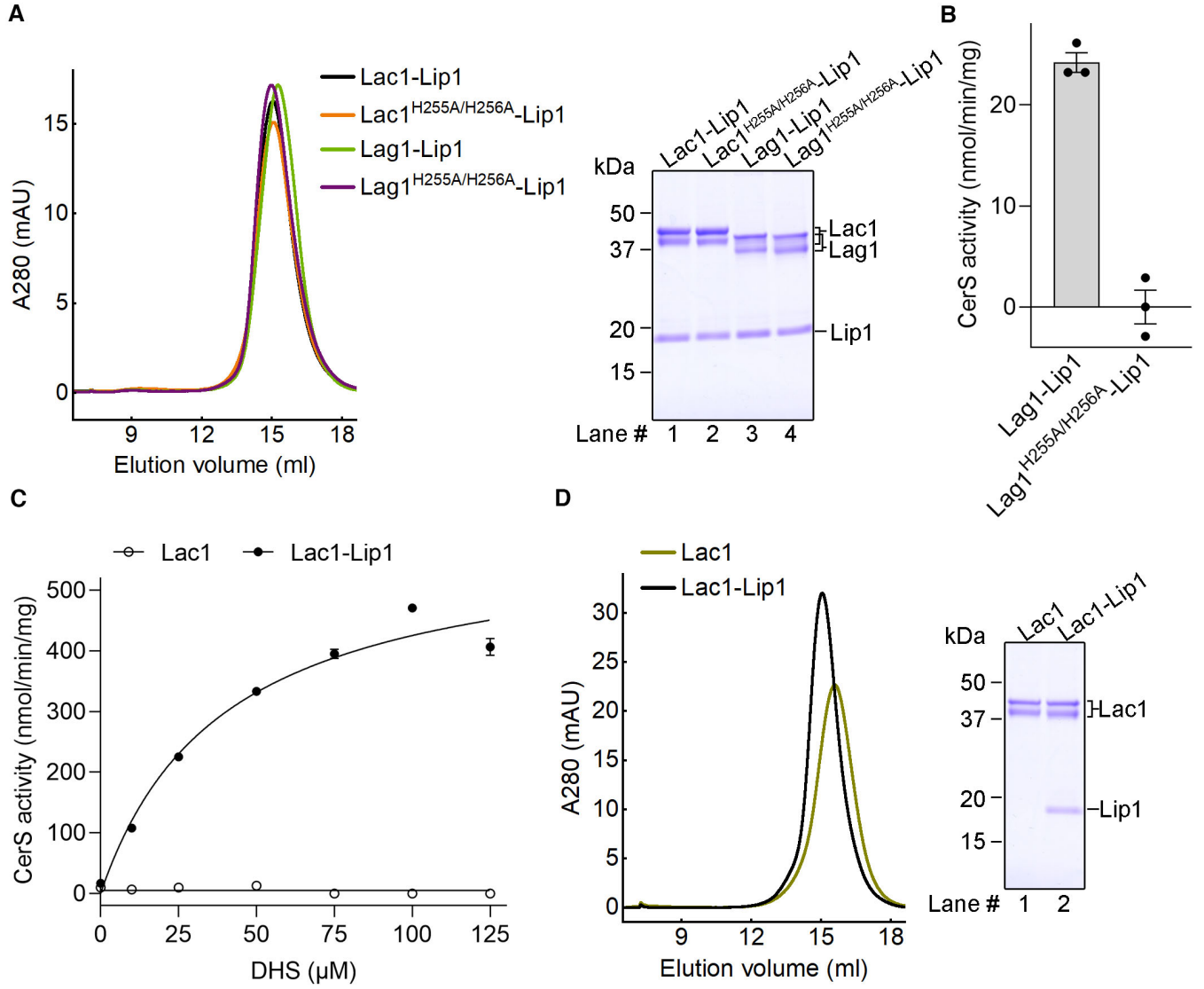

Figure EV2. Biochemical characterization of the Lac1-Lip1 and Lag1-Lip1 complexes.

A Size exclusion chromatography (SEC) profiles and Coomassie blue-stained SDS-PAGE gel of the purified Lac1-Lip1 and Lag1-Lip1 complexes.

B Enzymatic activity of the WT Lag1-Lip1 complex and the catalytic mutant Lag1$^{H255A/H256A}$-Lip1. Each data point is the average ± SEM of three independent experiments.

C CerS activity versus DHS concentration for the Lac1 alone protein and the Lac1-Lip1 complex. The activity curve of the Lac1-Lip1 complex follows a Michaelis–Menten equation with a K_m of 39.44 ± 6.29 μM for DHS and a V_{max} of 593.3 ± 34.5 nmol/min/mg. Each data point is the average ± SEM of three independent experiments.

D SEC profile and Coomassie blue-stained SDS-PAGE gel of the Lac1 alone protein and the Lac1-Lip1 complex.

Figure EV3. Features of Lip1 dimer.

A Overall structure of Lip1 dimer.

B Lip1 dimer interface.

C SEC profiles and Coomassie blue-stained SDS-PAGE gel of the Lip1 cysteine mutants.

D Normalized CerS activity of the Lip1 cysteine mutants. Each data point is the average ± SEM of three independent experiments.

E SEC profile and Coomassie blue-stained SDS-PAGE gel of the Lip1 dimer interface variant.

F Normalized CerS activity of the Lip1 dimer interface variant. Each data point is the average ± SEM of three independent experiments.

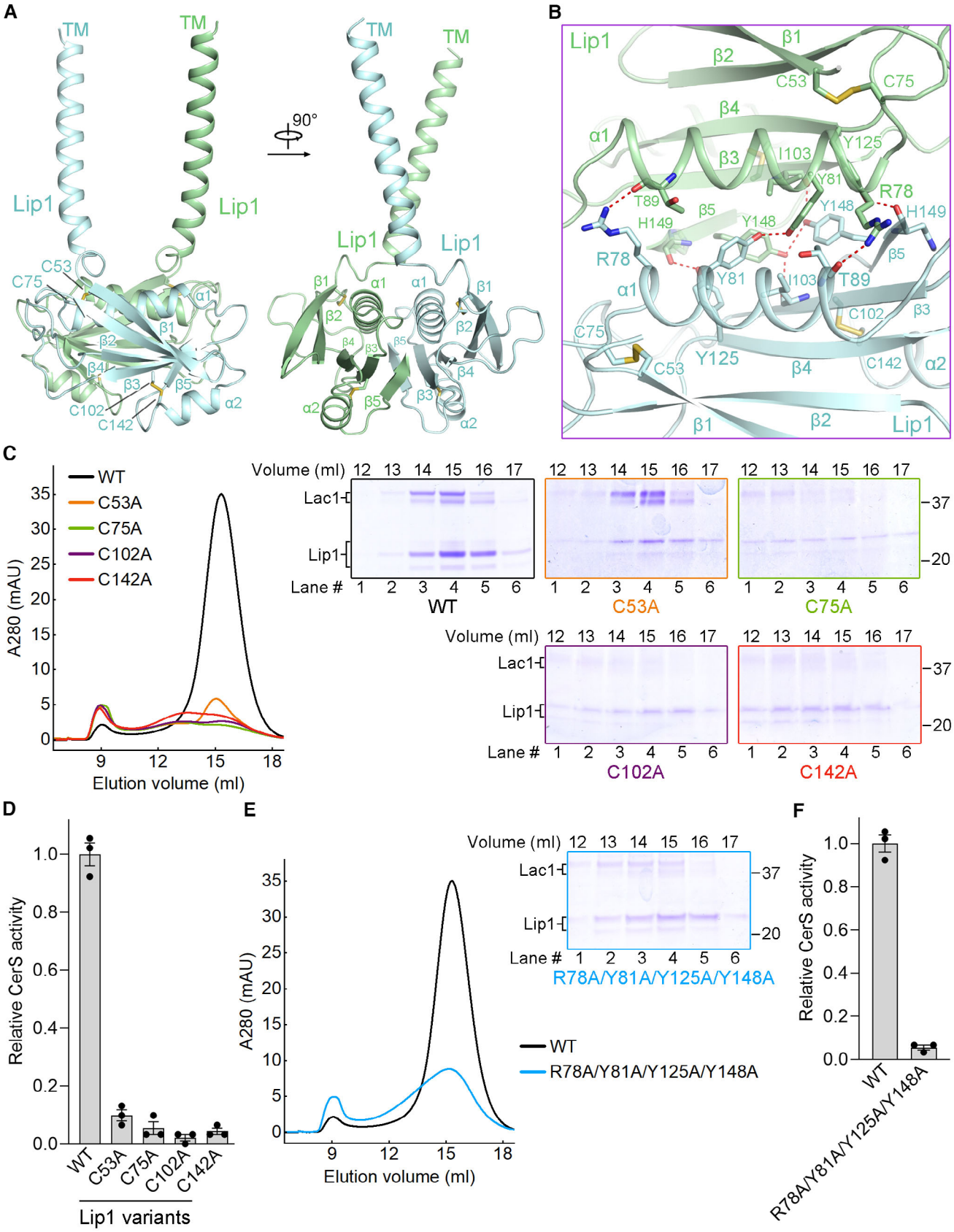
A
TM
TM
Lip1
Lip1
C53
C75
α1
β1
β2
β4
β3
β5
C102
C142
α2
90°
B
Lip1
β1
β2
C53
C75
β4
α1
β3
I103
Y125
Y81
R78
T89
β5
Y148
H149
C102
C142
α2
C
WT
C53A
C75A
C102A
C142A
A280 (mAU)
Elution volume (ml)
Volume (ml)
Lac1
Lip1
Lane #
37
20
D
Relative CerS activity
Lip1 variants
E
R78A/Y81A/Y125A/Y148A
F

Figure EV3.

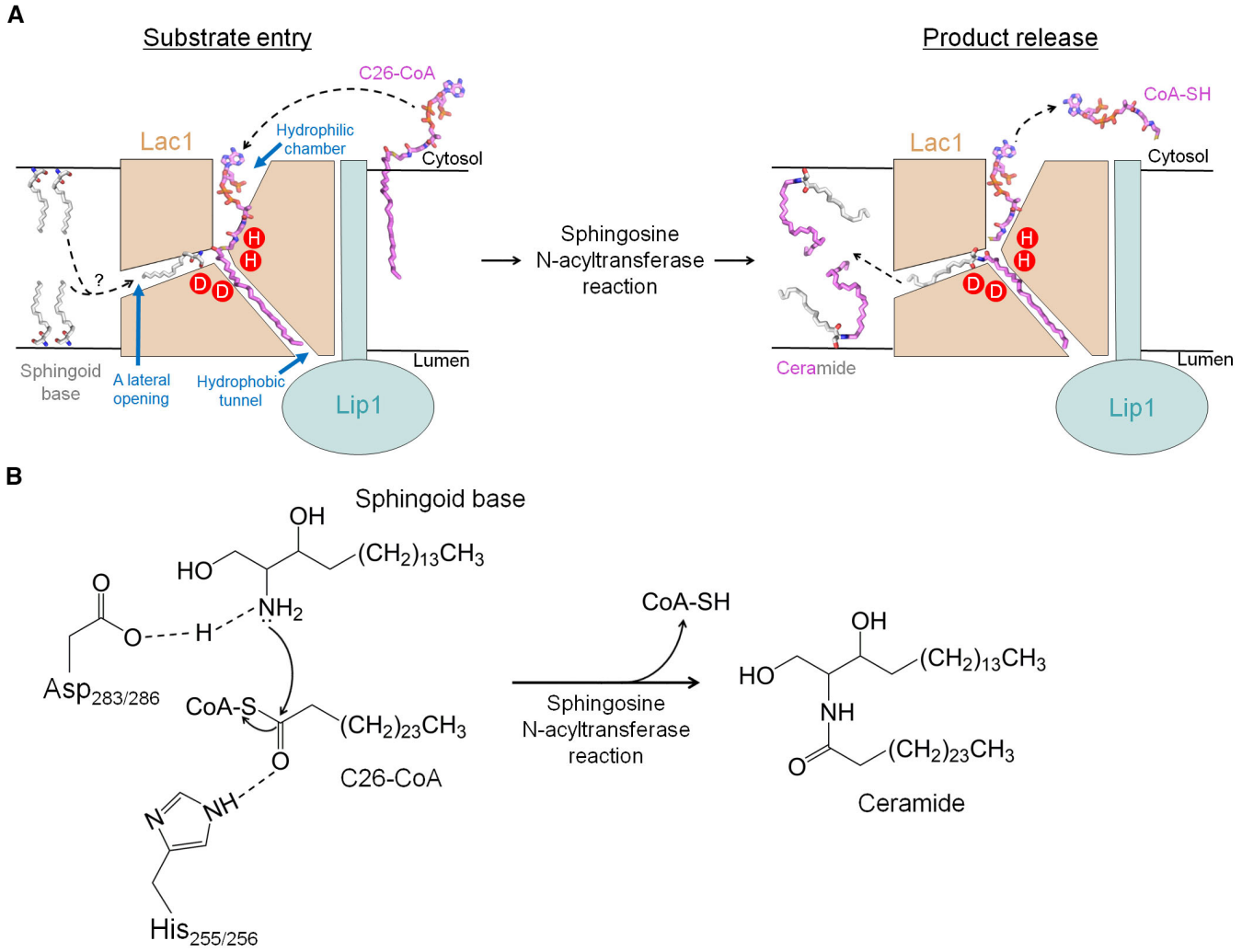

Figure EV4. Proposed working mechanism of yeast CerS.

A A hypothetic model for yeast CerS-catalyzed ceramide formation. The C26-CoA substrate enters the reaction chamber within Lac1 probably through the cytosolic side of the membrane. The CoA moiety of C26-CoA is located in the hydrophilic reaction chamber, while the acyl-chain moiety is positioned in the hydrophobic tunnel. Once the sphingoid base substrate from either leaflet of the membrane enters a lateral opening on Lac1, it approaches the catalytic histidine and aspartate residues near the thioester bond of C26-CoA, preparing for the sphingosine N-acyltransferase reaction. After the reaction, the product of free CoA-SH is released to the cytosol, whereas the hydrophobic product ceramide is released to either leaflet of the membrane. For simplicity, only one Lac1-Lip1 heterodimer is illustrated.

B Proposed catalytic mechanism. Asp283 or Asp286 of Lac1 functions as a general base to activate the amino group of the sphingoid base for nucleophilic attack on the carbonyl carbon of C26-CoA, which is coordinated by His255 or His256. The CerS-catalyzed acyl transfer reaction promotes the N-acylation of sphingoid base for ceramide production and releases free CoA-SH.

Figure EV5. Human CerS structural models predicted by AlphaFold.

A The AlphaFold-predicted human CerS structures (hCerS1AF-hCerS6AF). The N-terminal regions and Hox domains of hCerSs are colored light gray. The TLC domains are colored green with the acyl-CoA selective regions highlighted in dark pink. The predicted catalytic histidine and aspartate residues in the Lag1p motifs are shown in sticks.

B Structural superposition of the hCerS5AF (colored green) and the Lac1-Lip1 heterodimer (colored wheat and light cyan). The N-terminal region of hCerS5AF severely clashes with the TM of Lip1.

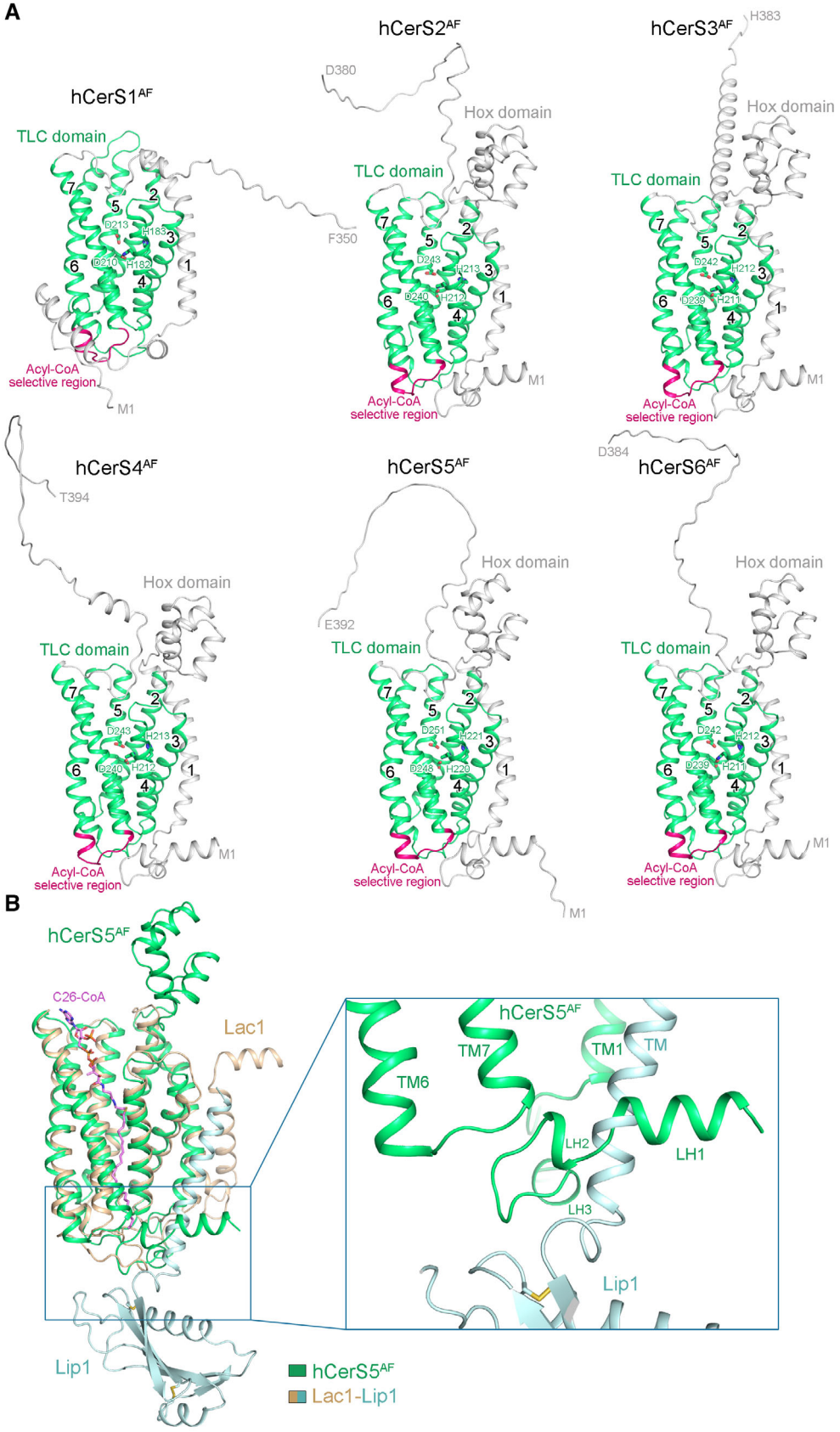
A
hCerS1AF
TLC domain
Acyl-CoA selective region
M1
hCerS2AF
D380
Hox domain
TLC domain
F350
Acyl-CoA selective region
M1
hCerS3AF
H383
Hox domain
TLC domain
Acyl-CoA selective region
M1
D384
hCerS4AF
T394
Hox domain
TLC domain
Acyl-CoA selective region
M1
hCerS5AF
Hox domain
E392
TLC domain
Acyl-CoA selective region
M1
hCerS6AF
Hox domain
TLC domain
Acyl-CoA selective region
M1
B
hCerS5AF
C26-CoA
Lac1
Lip1
hCerS5AF
Lac1-Lip1
TM7
TM1
TM
TM6
LH2
LH1
LH3
Lip1

Figure EV5.