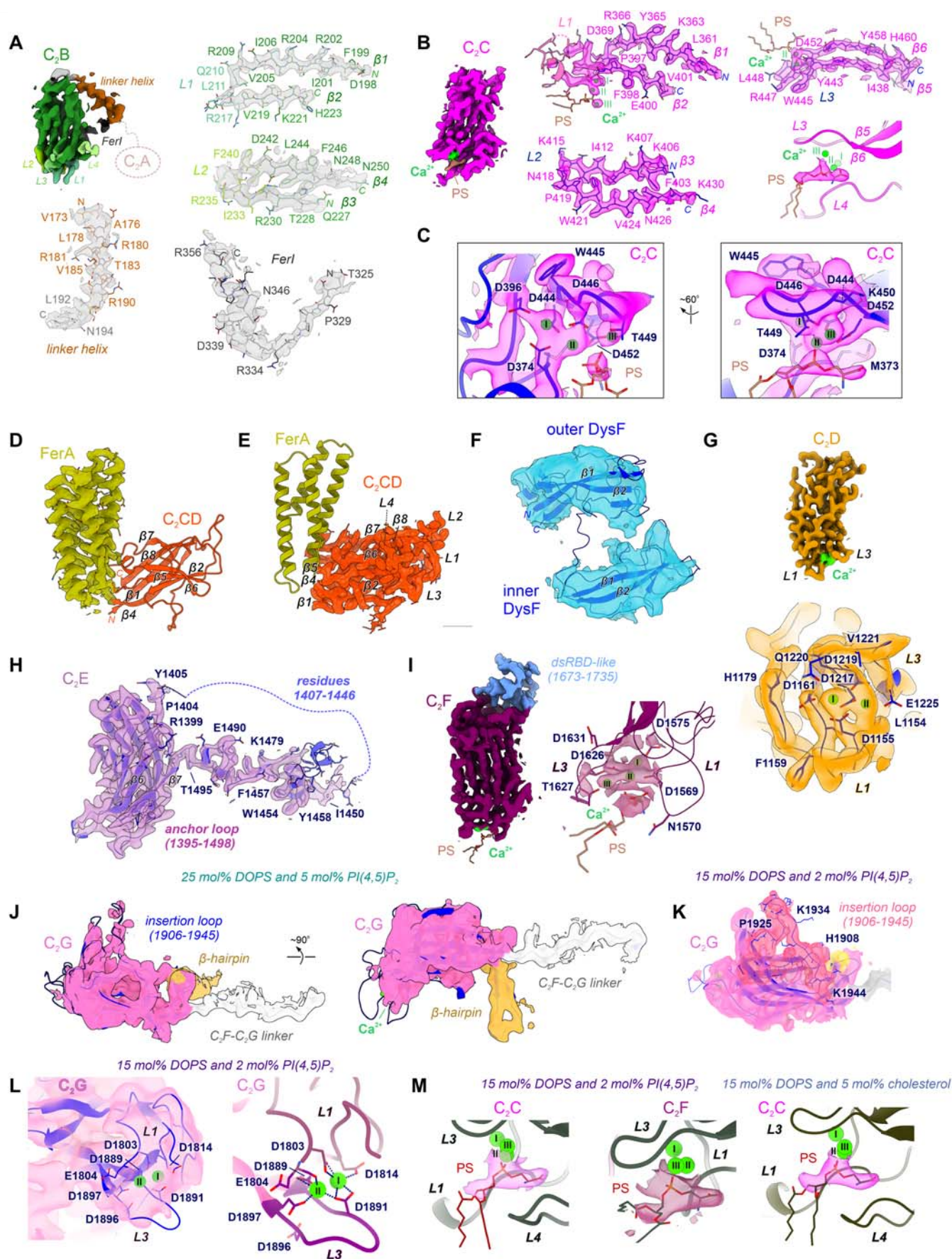
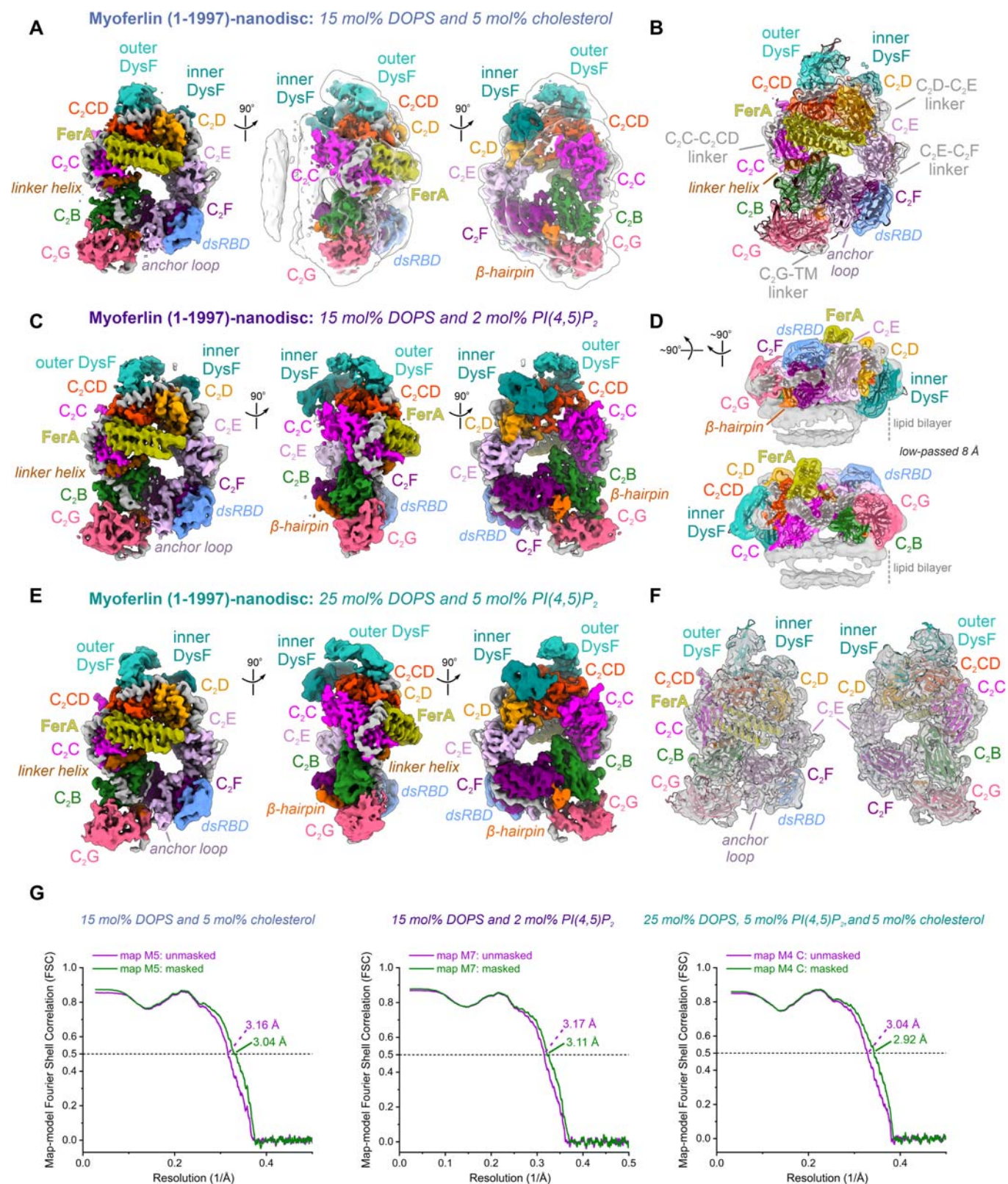


Expanded View Figures

Figure EV1. Cryo-EM density snapshots of lipid-bound soluble myoferlin (1-1997).

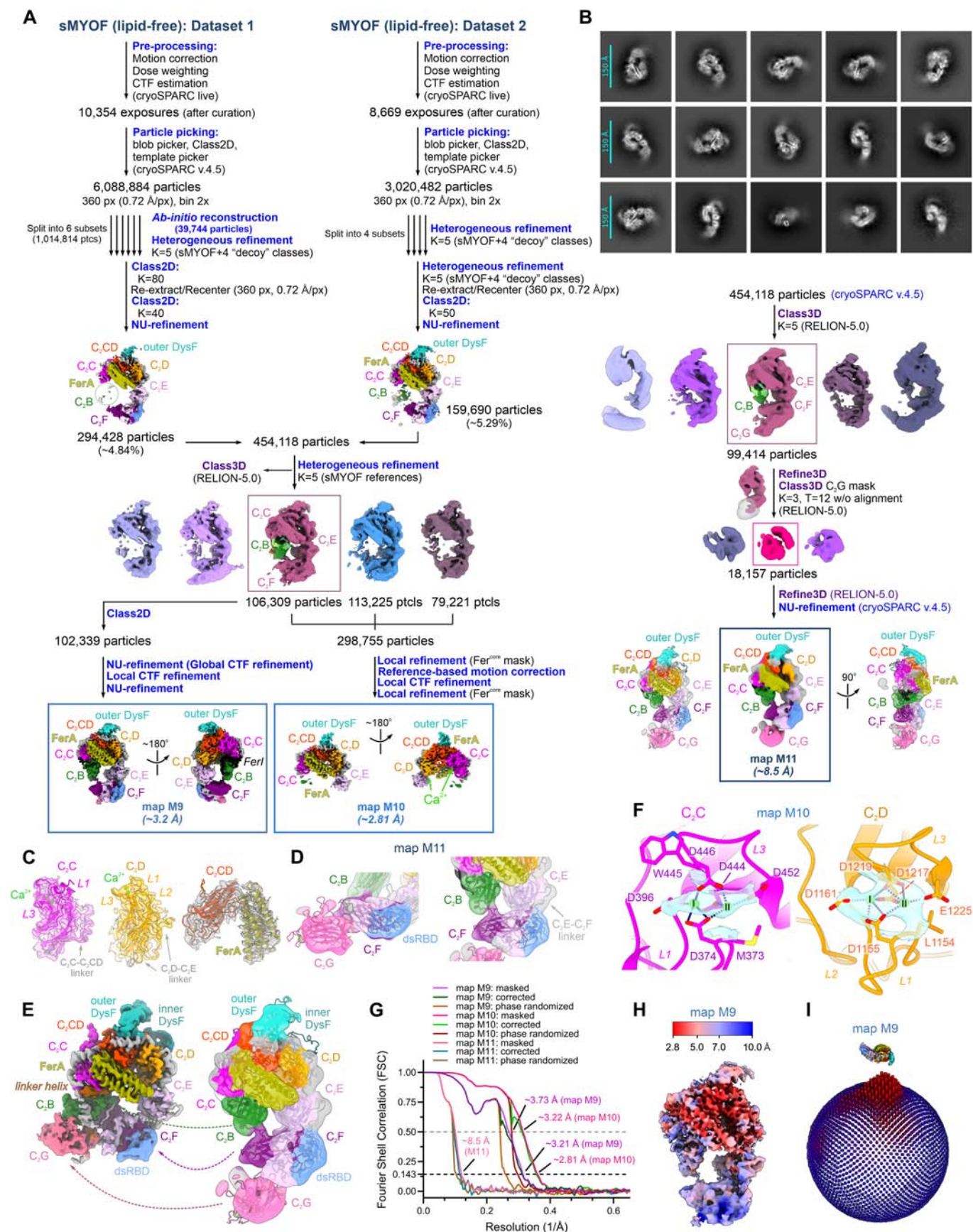
(A) Selected cryo-EM density snapshots of C₂B and its proximal motifs (the linker helix and the FerI motif). Myoferlin's residues are depicted as sticks. (B) Cryo-EM density of myoferlin's C₂C domain. The three bound Ca²⁺-ions and the recruited phosphatidylserine (PS) are indicated. (C) Ca²⁺-binding sites of the C₂C domain of myoferlin. The cryo-EM density is contoured around the Ca²⁺-binding residues of C₂C. (D) Cryo-EM density of the FerA motif of myoferlin. Notably, the four-helix bundle domain inserts between the β 4 and β 5 strands of the C₂CD domain (red, shown in a cartoon representation). (E) Cryo-EM density of the C₂CD domain of myoferlin. The seven β -strands of the domain are indicated. (F) Cryo-EM densities of the modelled inner and outer DysF motifs of myoferlin. (G) Cryo-EM density and the observed Ca²⁺-binding sites of the C₂D domain of myoferlin. C₂D's cryo-EM density is contoured around the Ca²⁺-binding sites (bottom panel). (H) Density of the C₂E domain. The domain comprises an extended insertion loop (residues 1395-1498, denoted as the anchor loop), inserted between the β 6- β 7 strands. The loop establishes contacts with the downstream C₂F domain. (I) Cryo-EM density of C₂F and its Ca²⁺- and phospholipid-binding sites. Three Ca²⁺ ions and a PS headgroup were identified in the cryo-EM density map. (J) Cryo-EM density of the C-terminal C₂G domain, derived from the myoferlin (1-1997)-nanodisc complex containing 25 mol% DOPS and 5 mol% PI(4,5)P₂. The C₂G domain is shown in two orientations, with its lipid-binding β -hairpin motif coloured light orange. (K) Density of the C-terminal C₂G domain, resolved in the myoferlin (1-1997)-nanodisc complex containing 15 mol% DOPS and 2 mol% PI(4,5)P₂. Notably, the insertion loop of C₂G (residues 1906-1944) is ordered in this myoferlin complex. (L) Ca²⁺-binding sites of C₂G, observed in the lipid-bound myoferlin structure (15 mol% DOPS and 2 mol% PI(4,5)P₂ nanodisc). Due to the lower local resolution, Ca²⁺ modelling was initiated by AlphaFold3 predictions (Abramson et al, 2024), and the optimal sites were refined against the cryo-EM map of the complex (Appendix Fig. S5F). Our modelling (right panel) suggests that two Ca²⁺ ions are coordinated by the L1 and L3 loops of C₂G (Corbalan-Garcia and Gomez-Fernandez, 2014; Rizo and Sudhof, 1998). (M) Cryo-EM densities of the Ca²⁺-bound phosphatidylserine (PS), resolved in the myoferlin-nanodisc complexes. The cryo-EM density is contoured around the PS headgroups, and the ligands are depicted as sticks.





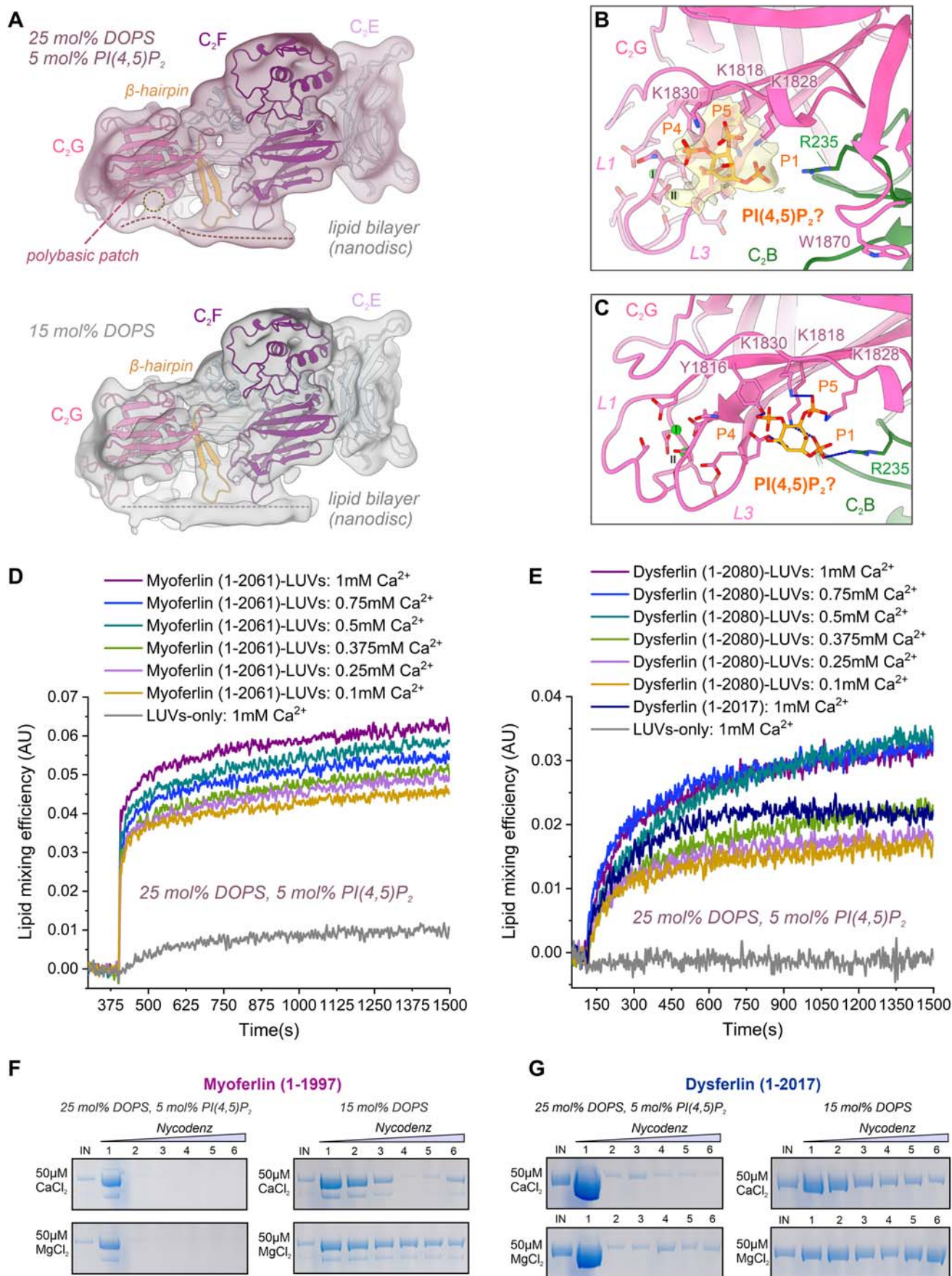
◀ **Figure EV2. Structural comparison between the different lipid-bound myoferlin complexes.**

(A) Overall cryo-EM map (map M6, ~ 3.3 Å) of soluble myoferlin (1-1997) bound to an MSP2N2 nanodisc containing 15 mol% DOPS and 5 mol% cholesterol (Appendix Fig. S5C). The map is shown in three different orientations. A lowpass filtered map (transparent surface) is superimposed to better visualize the nanodisc density. (B) Cryo-EM map of the myoferlin (1-1997)-nanodisc complex (15 mol% DOPS and 5 mol% cholesterol nanodisc). The final model is fitted inside the map (Appendix Fig. S5C). Several C₂ domain linker regions, the linker helix, and the anchor loop of C₂E are well resolved in this myoferlin complex. (C) Overall cryo-EM map (map M8, ~ 3.43 Å) of the soluble myoferlin (1-1997) bound to a nanodisc containing 15 mol% DOPS and 2 mol% PI(4,5)P₂ (Appendix Fig. S5F). The map is shown in three different orientations, as in (A). (D) Side views of the myoferlin (1-1997)-nanodisc complex (map M8, 15 mol% DOPS and 2 mol% PI(4,5)P₂), together with the fitted model (Appendix Fig. S5F). The map was lowpass filtered to 8 Å, and the nanodisc density is indicated. (E) Cryo-EM map of the myoferlin (1-1997)-nanodisc complex assembled onto a 25 mol% DOPS and 5 mol% PI(4,5)P₂ MSP2N2 nanodisc (~ 2.56 Å, map M3, Appendix S2C). (F) Overall map of the nanodisc-bound myoferlin (1-1997) complex (map M3, 25 mol% DOPS and 5 mol% PI(4,5)P₂) with the fitted final model. Except for the flexible N-terminal C₂A, all myoferlin domains (C₂B-C₂G) and accessory motifs (FerA, the linker helix, the anchor loop) were accurately built, resulting in the near-complete structure of ferlin's cytosolic region (Appendix Figs. S2C and S3). (G) Map versus model Fourier Shell Correlation (FSC) plots for the myoferlin (1-1997)-nanodisc complexes (Appendix Figs. S4A and S5C,F).



◀ **Figure EV3. Cryo-EM image analysis of the vitrified lipid-free soluble myoferlin (1-1997).**

(A) Cryo-EM image processing schematic for the Ca^{2+} -bound, lipid-free myoferlin (1-1997). The resolution of the final maps (M9, M10, and M11 maps) was estimated according to the gold-standard Fourier Shell Correlation (FSC) criterion of 0.143. The structural domains of myoferlin are colour-coded as in Fig. 1. (B) Reference-free 2D class averages of lipid-free myoferlin (1-1997). Note the similarities between the dysferlin (1-2017) (Appendix Fig. S6C) and myoferlin (1-1997) 2D class averages in their lipid-free states. (C) Cryo-EM density of myoferlin's C_2C , C_2D , and C_2CD -FerA domains. The myoferlin model is fitted inside, and the Fer^{core} map of myoferlin (map M10) is shown as a transparent surface. (D) Cryo-EM density of the C_2F (map M9) and C_2G (map M11) domains as modelled in the lipid-free myoferlin (1-1997) structure. The tertiary interfaces between the C_2F - C_2B and C_2F - C_2G are also observed in the lipid-free dysferlin (1-2017) structure (Appendix Figs. S6A, S7G-J, and S8H,I). (E) Side-by-side comparison between the membrane-bound (map M3) and lipid-free (map M11) myoferlin structures. The domains undergoing significant displacement upon nanodisc binding (C_2B , C_2F , and C_2G) are indicated with dashed arrows. (F) Ca^{2+} -binding sites observed in the lipid-free myoferlin structure. The cryo-EM density is coloured cyan and shown as a transparent surface. The two modelled Ca^{2+} ions, bound to C_2C and C_2D , are coloured green. (G) Global resolution estimates for the lipid-free myoferlin (1-1997) cryo-EM maps using Fourier Shell Correction (FSC) between half-maps. The resolution estimates at the $\text{FSC} = 0.143$ and $\text{FSC} = 0.5$ thresholds are indicated. (H) Local resolution of the overall map of the lipid-free soluble myoferlin (map M9). The map regions coloured in red indicate higher resolution. (I) Angular distribution of the myoferlin particles contributing to the overall map (map M9). The final cryo-EM map is shown above the 3D angular distribution representation. Relative cylinder height and the red colour indicate a higher number of particle images. Source data are available online for this figure.



◀ **Figure EV4. Full-length myoferlin and dysferlin appear to promote tight binding between vesicles.**

(A) Comparison of the local nanodisc structures observed in the myoferlin (1-1997)-nanodisc complexes. The lipid nanodisc forms close contacts with the concave surface of C₂G in the presence (top, 25 mol% DOPS, 5 mol% PI(4,5)P₂, and 5 mol% cholesterol nanodisc, Appendix Fig. S4A-E), but not in the absence of PI(4,5)P₂ (bottom, 15 mol% DOPS and 5 mol% cholesterol nanodisc, Appendix Fig. S5C). The cryo-EM maps have been lowpass filtered to 10 Å, and the myoferlin model is fitted inside. (B) The cryo-EM density element proximal to C₂G could accommodate a PI(4,5)P₂ headgroup. C₂G and C₂B residues located at the interface are displayed as sticks. The putative PI(4,5)P₂ density lies in close proximity to three lysine residues (K1818, K1828, and K1830), projecting from the concave surface of C₂G, as well as to an arginine residue of C₂B (R235). The Ca²⁺-binding L3 loop of C₂G is also in close proximity. (C) Polar contacts between several basic residues of C₂G and C₂B and a tentative phospholipid, possibly PI(4,5)P₂, originating from the nanodisc bilayer. The nanodisc contained 25 mol% DOPS and 5 mol% PI(4,5)P₂. (D) Lipid mixing assays between myoferlin proteoliposomes and PS/PI(4,5)P₂-bearing vesicles in the presence of varying Ca²⁺ concentrations. The fluorescent liposomes (Large unilamellar vesicles (LUVs), 25 mol% DOPS and 5 mol % PI(4,5)P₂) were dual-labelled with the Lissamine Rhodamine B and NBD (Nitrobenzoxadiazole). The NBD dequenching signal was used to monitor the extent of tight vesicle-vesicle docking (and, possibly, fusion) to non-fluorescent proteoliposomes. LUVs lacking full-length myoferlin (1-2061) were used as a control. Fluorescence traces were smoothed (using the Savitzky-Golay method) and normalized to the maximal dequenching signal to calculate the lipid mixing efficiency. The initial fluorescence increase is likely due to ferlin-induced aggregation of proteoliposomes. The lipid mixing assays were performed in triplicate, and representative fluorescence traces are shown. (E) Lipid mixing assays between dysferlin proteoliposomes and PS/PI(4,5)P₂-containing vesicles. The ability of full-length dysferlin (1-2080) to promote tight vesicle-vesicle docking was assessed as a function of Ca²⁺ concentration. The lipid mixing activity of soluble dysferlin (1-2017) was also tested (deep blue). Empty LUVs were used as a control. All assays were repeated at least three times, and representative fluorescence traces are shown. (F, G) Ca²⁺-dependent liposome binding activity of soluble myoferlin (1-1997) and dysferlin (1-2017) assessed using a coflotation assay. The liposomes used in these assays had a similar lipid composition to those in (D, E). The Nycodenz step gradients (0%/30%/40%) were harvested from the top and analysed by SDS-PAGE. Both myoferlin (1-1997) and dysferlin (1-2017) showed increased binding to LUVs containing both DOPS and PI(4,5)P₂. Source data are available online for this figure.