Supplemental Information (SI) Appendix

Structural analysis of a trimeric assembly of the mitochondrial dynamin-like GTPase Mgm1

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Includes:

SI figures, Fig S1-S7

SI tables, Table S1-S2

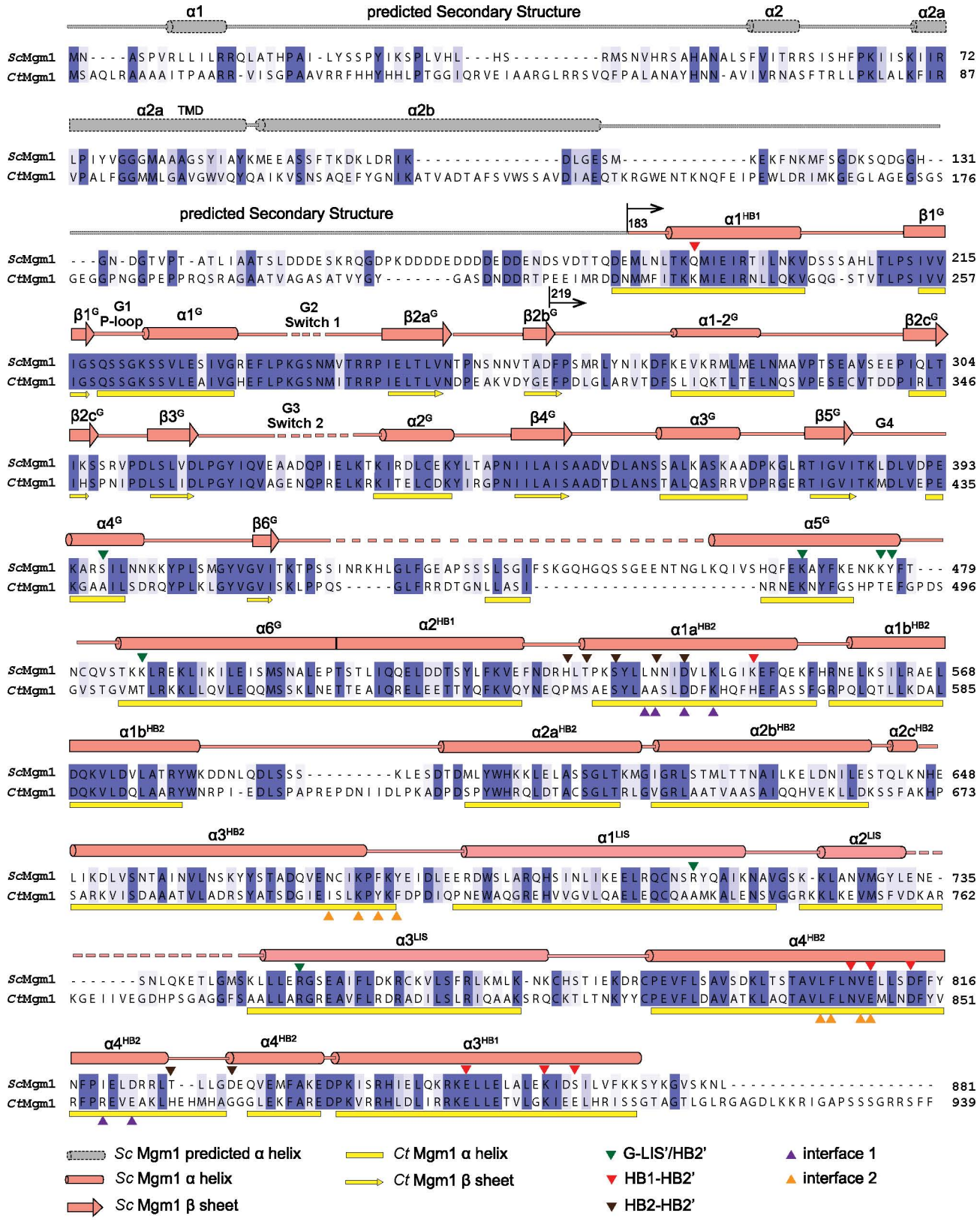

Figure S1. Sequence alignment of Mgm1 and OPA1

Sequences of *S. cerevisiae* (*Sc*) Mgm1and *C. thermophilum* Mgm1 (*Ct*Mgm1) are numbered and aligned. The predicted and observed secondary structures of Mgm1 are labelled and coloured as indicated. Signature motifs of the G domain and residues at critical interfaces are highlighted.

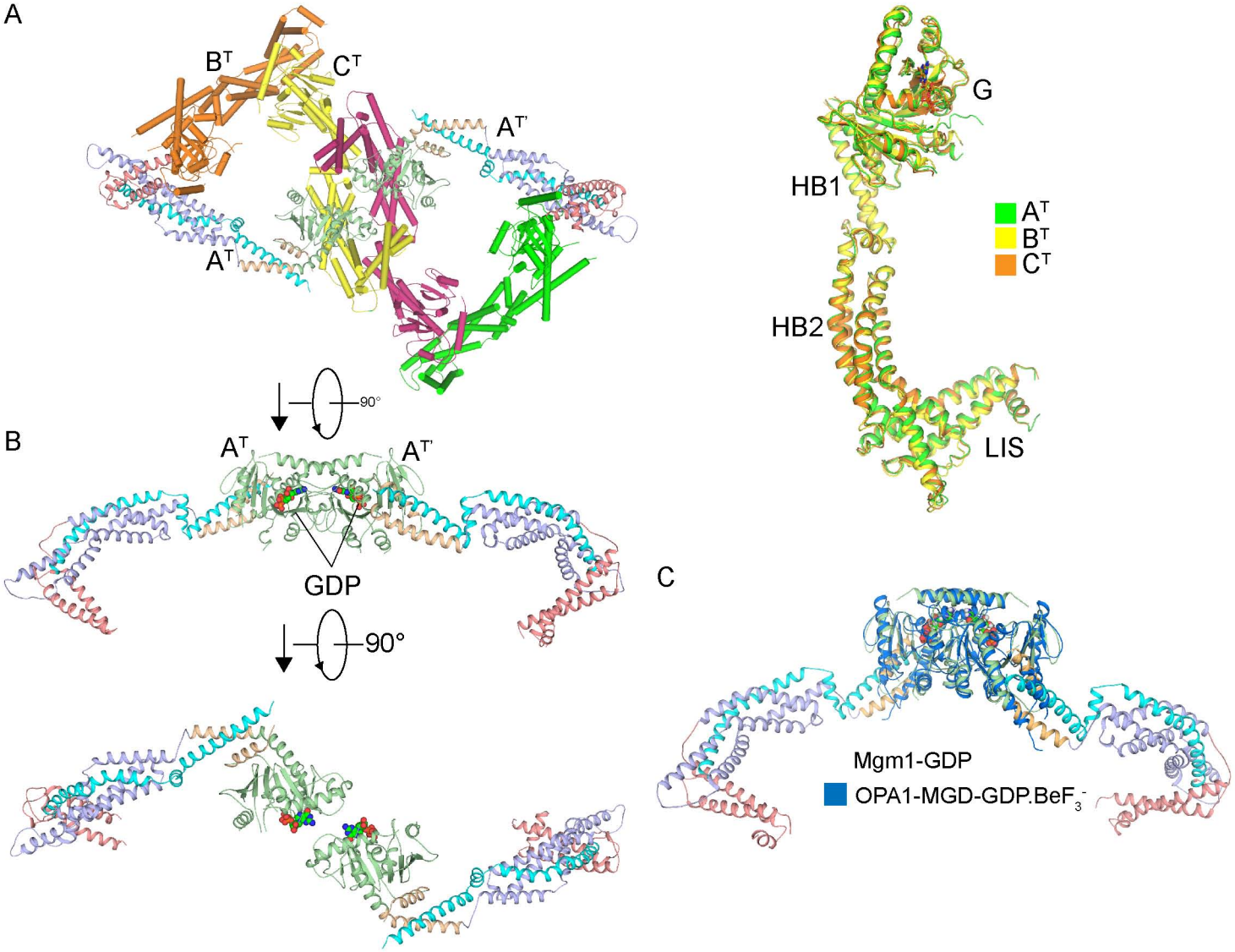

Figure S2. Structural comparison of Mgm1 and OPA1
(A) Two neighbouring asymmetric units are shown. The linking molecules are shown in cartoon representation. On the right, three protomers in each asymmetric unit are labelled A, B, and C and superimposed. The main domains are highlighted. (B) The two linking molecules between asymmetric units are shown individually and viewed from two angles. Notably, no substantial interactions were seen at the G-G interface. (C) Two s-Mgm1 molecules are superimposed onto the OPA1-MGD dimer.

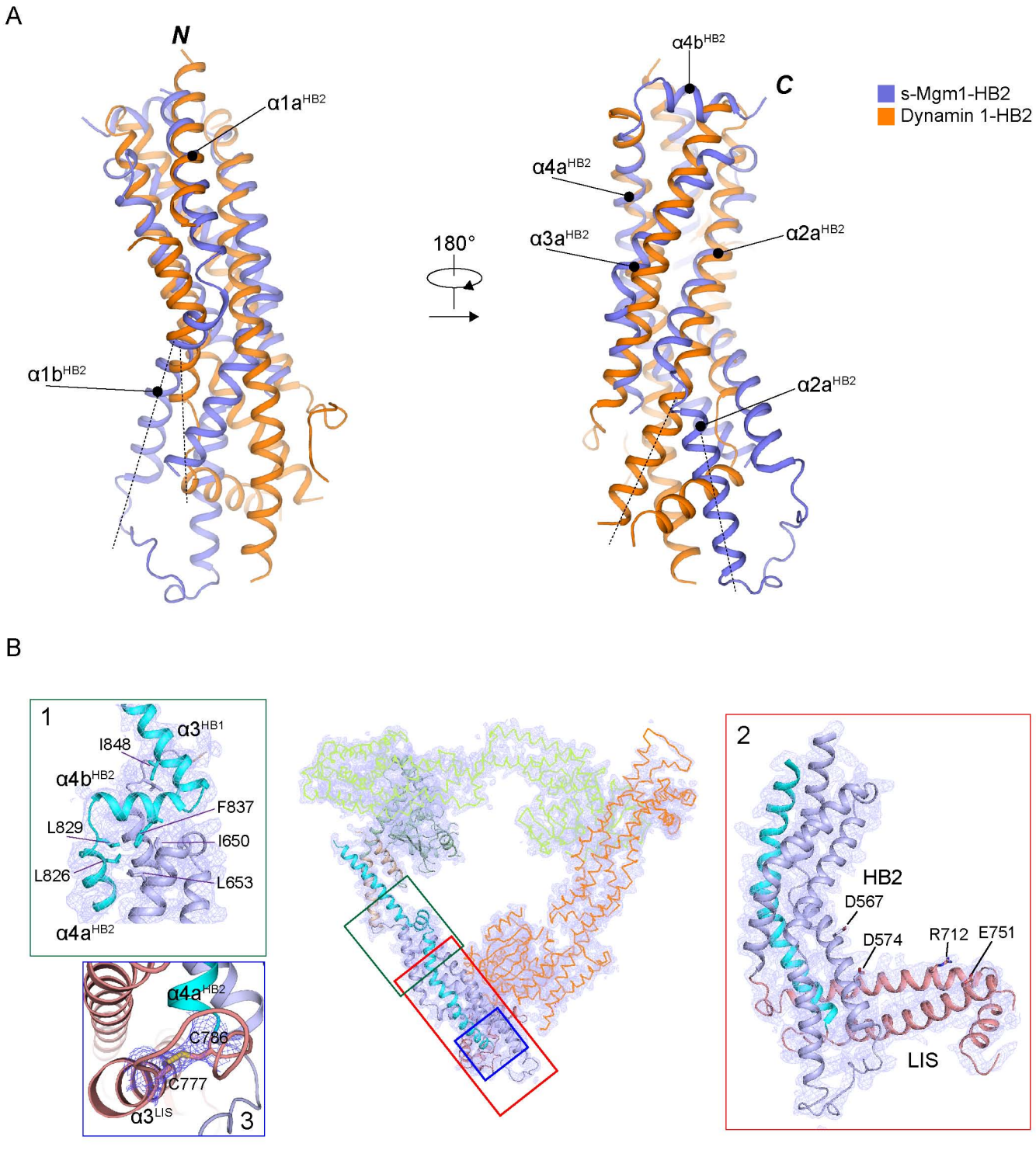

Figure S3. Structure of the helical domains in s-Mgm1
(A) The HB2 domain of s-Mgm1 is superimposed with the stalk domain of dynamin-1 (PDB code: 3SNH). Helices of s-Mgm1 are labelled. Deviating angles of helices in the two structures are highlighted. (B) The 2Fo - Fc electron density maps (1.0σ contour) of the s-Mgm1 trimer are shown as wire mesh (blue). Three boxed regions are shown with zoomed-in views and key residues highlighted in a stick representation.

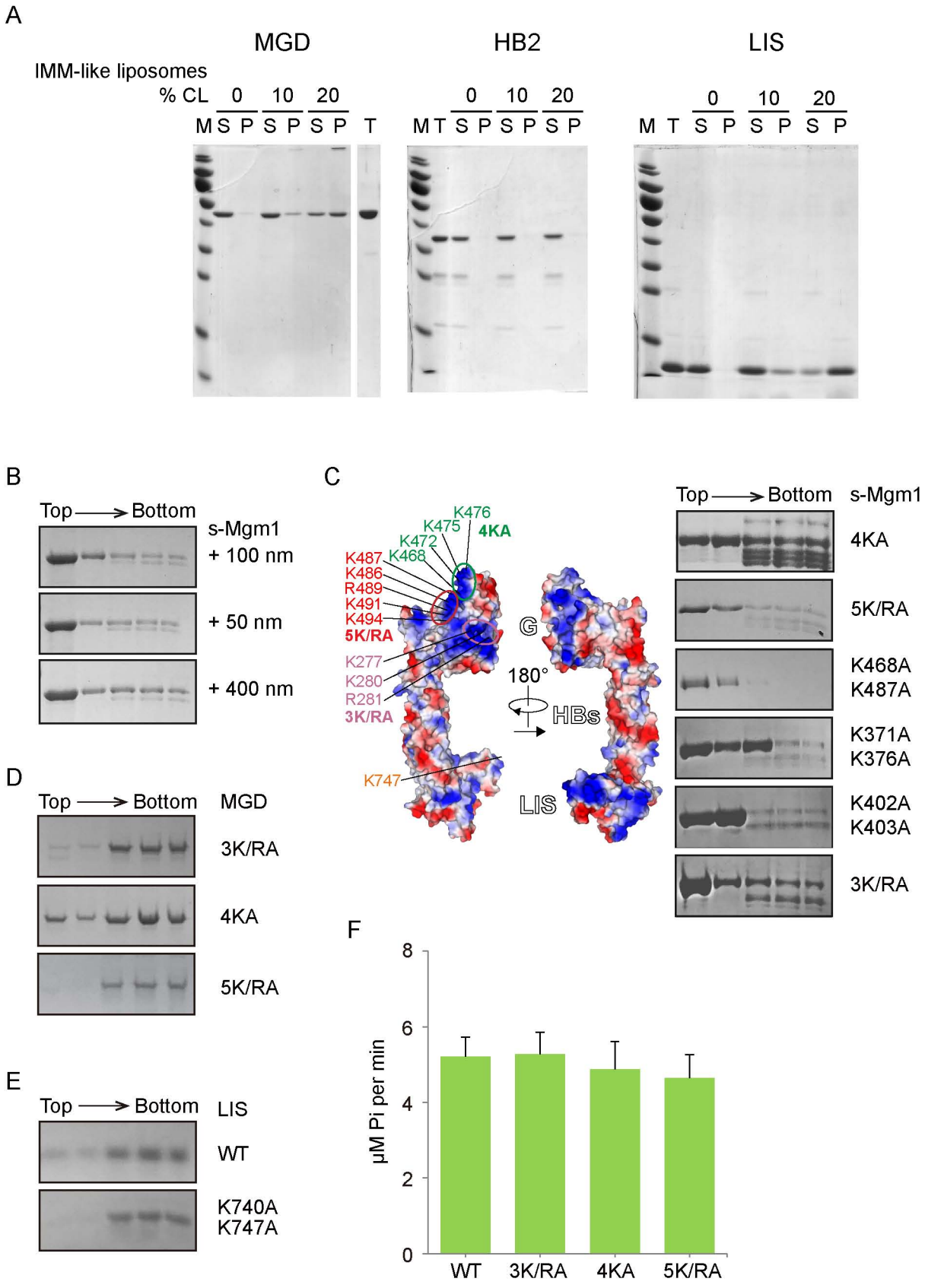

Figure S4. Lipid interactions of s-Mgm1

(A) Different regions of s-Mgm1 were purified and subjected to the liposome pelleting assay. Inner membrane-mimicking liposomes with the indicated cardiolipin (CL) percentages were used. The data are representative of at least three repetitions. M, molecular marker; S, supernatant; P, pellet; T, total loaded protein. (B) Liposome flotation assay as in Fig. 2F, but with liposomes of various sizes. (C) Electrostatic analysis of s-Mgm1. Negatively charged surfaces are coloured in red and positively charged surfaces in blue. The positions of mutated residues are highlighted. Flotation assays of s-Mgm1 mutants are shown on the right. (D) As in B, but with purified MGD mutants. (E) As in B, but with purified LIS mutants. All flotation data are representative of at least three repetitions. (F) The GTPase activity of various Mgm1-MGD constructs was measured in the presence of 500 mM KCl and 5 mM $MgCl_2$. A total of 5 µM of protein was used for each sample. GTP hydrolysis was measured by phosphate release at saturating GTP concentrations (0.5 mM). Data are presented as the mean ± s.d. of nine measurements from three independent experiments.

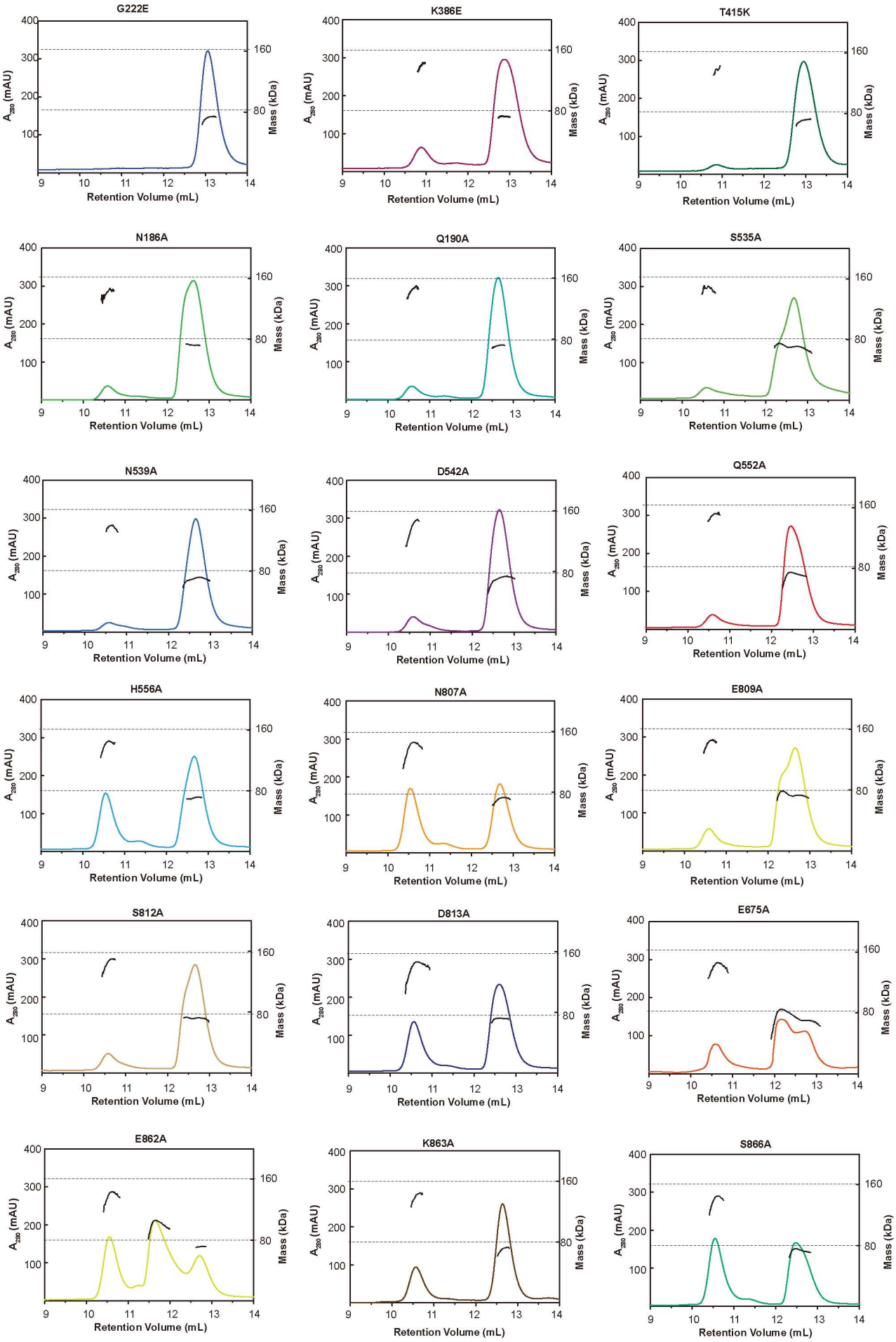

Figure S5. Dimerization of s-Mgm1 mutants

The size of s-Mgm1 (theoretical molecular mass 80.0 kDa) was determined by MALS coupled with gel filtration in the absence of nucleotides in a buffer containing 500 mM KCl. The estimated molecular masses are shown by the right axis. The data are representative of at least three repetitions.

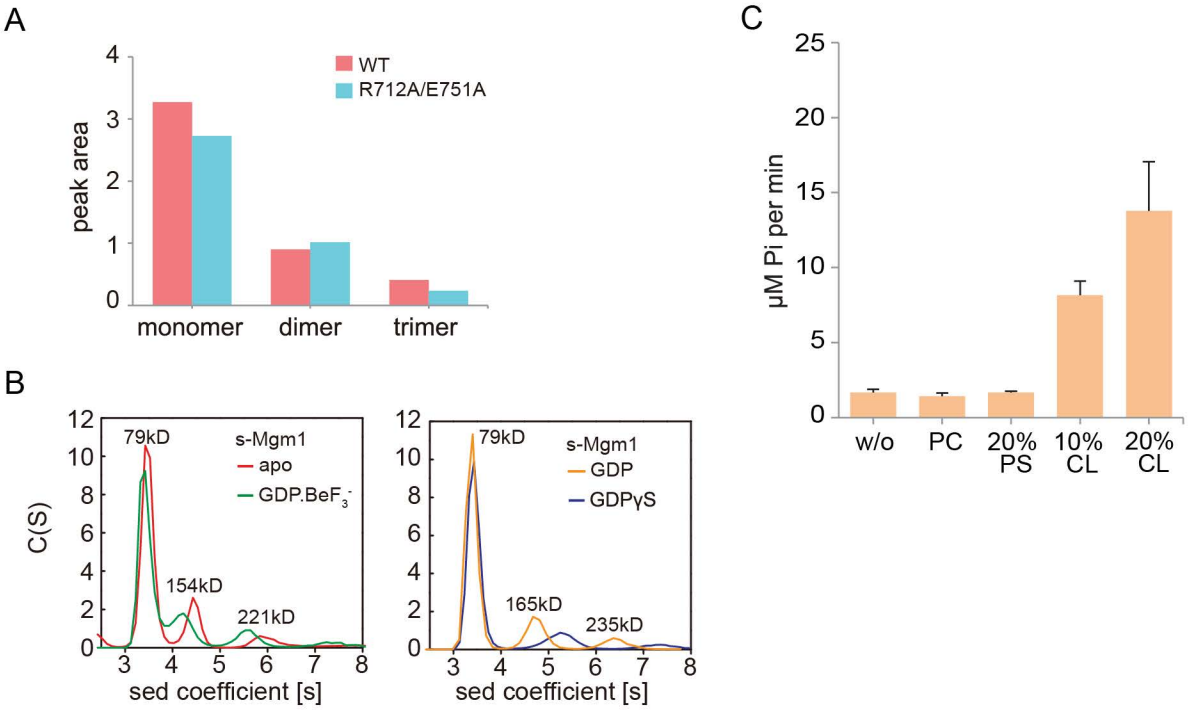

Figure S6. Assembly of s-Mgm1

(A) The relative peak areas of results shown in Fig. 4A. (B) The size of s-Mgm1 (theoretical molecular mass 80.0 kDa) was determined at 25 µM by analytical ultracentrifugation (AUC) in the absence or presence of 0.5 mM GDP and 2.5 mM BeF_3^- in a buffer containing 500 mM KCl. The estimated molecular masses are given above the peaks (in kilodaltons, kDa). The data are representative of at least three repetitions. (C) The GTPase activity of s-Mgm1 constructs was measured in the absence or presence of liposomes with the indicated contents in a buffer with 100 mM KCl and 5 mM $MgCl_2$. A total of 1 µM of protein was used for each sample. GTP hydrolysis was measured by phosphate release at saturating GTP concentrations (0.5 mM). Data are presented as the mean ± s.d. of nine measurements in three independent experiments.

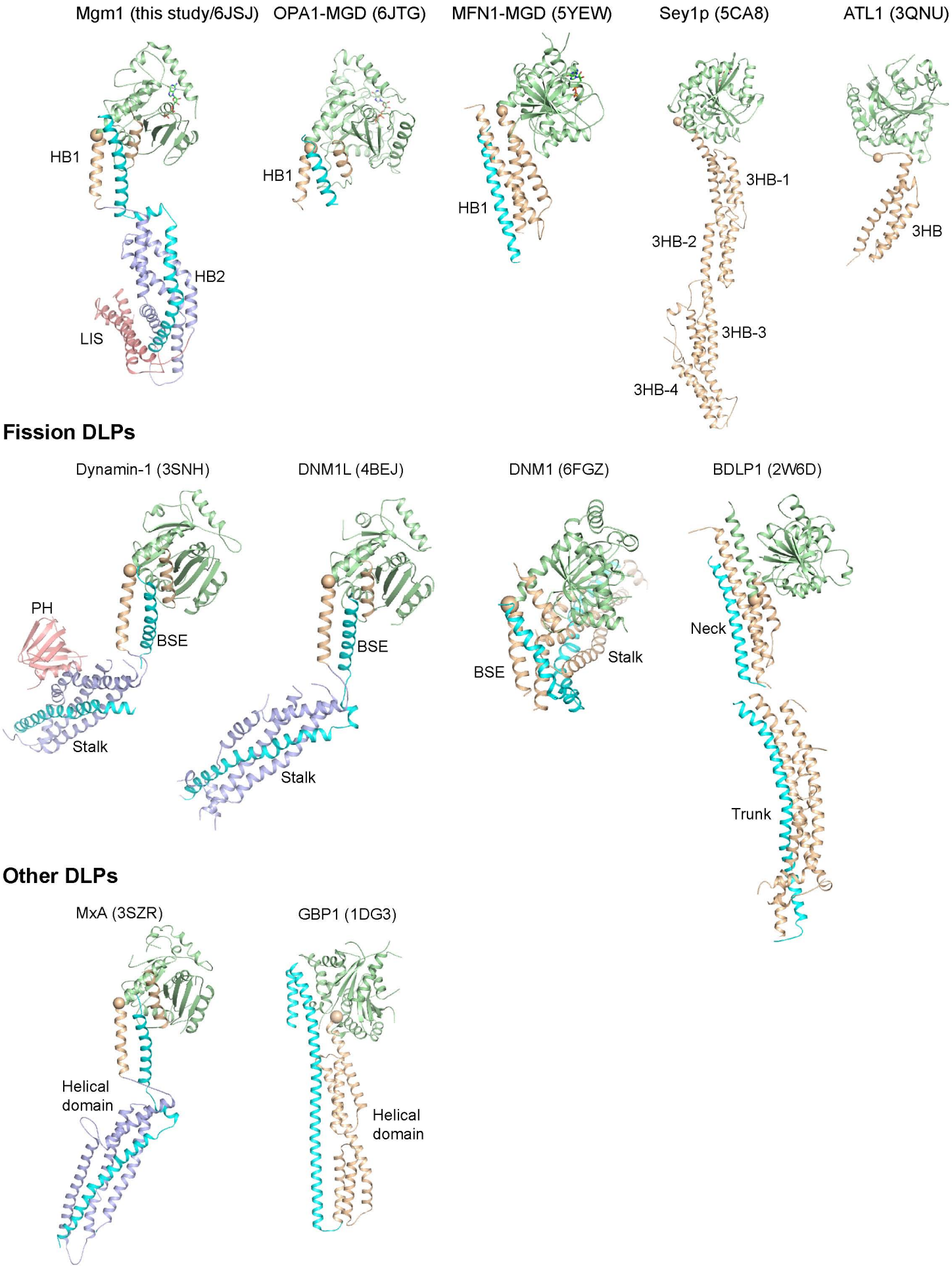

Figure S7. Structures of DLPs categorized by their membrane remodelling activities
PDB codes are indicated and key domains highlighted.

Table S1 | Data collection and refinement statistics

Parameters (Data collection statistics)	Se-Met s-Mgm1
Data collection statistics	
Cell parameters	
a (Å)	153.5
b (Å)	153.5
c (Å)	236.0
α, β, γ (°)	90.0, 90.0, 90.0
Space group	$P4_12_12$
Wavelength used (Å)	0.9798
Resolution (Å)	50.0–3.2 (3.32-3.20)[c]
No. of all reflections	37,364,35 (263,590)
No. of unique reflections	46,877 (3,404)
Completeness (%)	99.9 (100.0)
Average I/σ(I)	18.07 (1.29)
R_{merge}[a] (%)	16.8 (80.3)
Refinement statistics	
No. of reflections used (σ(F) > 0)	47,088
R_{work}[b] (%)	22.6
R_{free}[b] (%)	30.7
r.m.s.d. bond distance (Å)	0.026
r.m.s.d. bond angle (º)	2.998
Average B-value (Å^2)	95.5
No. of protein atoms	14,704
No. of ligand atoms	93
No. of solvent atoms	0
Ramachandran plot	
res. in favored regions (%)	91.66
res. in allowed regions (%)	7.39
res. in outlier regions (%)	0.95

[a] $R_{merge} = \Sigma_h\Sigma_l \mid I_{ih} - I_h \mid / \Sigma_h\Sigma_I I_h$ where I_h is the mean of observations I_{ih} of reflection h.

[b] $R_{work} = \Sigma(\mid\mid F_p(obs)\mid - \mid F_p(calc)\mid\mid) / \Sigma\mid F_p(obs)\mid$. R_{free} is an R factor for a pre-selected subset (5%) of reflections that was excluded in the refinement.

[c] Numbers in parentheses are corresponding values for the highest resolution shell.

Table S2 | Functional analysis of Mgm1 in yeast cells

		Tubular Morphology	Cells with mtDNA	Growth
WT W303		95.7 ± 1.5	94.5 ± 2.0	+
Δ*mgm1*		0.0 ± 0.0	0.0 ± 0.0	-
Δ*mgm1*	pRS425	0.2 ± 0.2	0.2 ± 0.2	-
	WT	83.2 ± 5.6	70.6 ± 9.7	+
	Δ149-173	83.2 ± 6.9	36.6 ± 7.2	partial +/-
	Δ161-173	29.7 ± 5.6	15.2 ± 3.0	patial +/- -
	Δ161-198	0.4 ± 0.4	1.4 ± 0.8	-
	Q190A	22.7 ± 7.7	25.2 ± 1.5	partial +/-
	Q190A E809A	14.9 ± 3.7	8.6 ± 9.0	-
	R195E	0.4± 0.4	2.6 ± 2.3	-
	Q219R	3.0 ± 0.8	3.3 ± 3.3	-
	S220A	0.6 ± 0.6	0.0 ± 0.0	-
	KGS238DDA	0.5 ± 0.5	0.0 ± 0.0	-
	K238D	2.4 ± 1.6	0.0 ± 0.0	-
	G239D	71.2 ± 9.3	63.7 ± 15.1	partial +/-
	S240A	79 ± 5.4	78.6 ± 1.9	+
	V324D	79 ± 4.7	5.5 ± 1.0	partial +/-
	D360A	15.6 ± .41	12.0 ± 2.7	partial +/-
	L367D	16.5 ± 3.4	10.8 ± 2.7	partial +/-
	S397A	68.2 ± 3.1	43.7 ± 4.4	+
	S397A E751A	30.5 ± 9.0	0.0 ± 0.0	-
	Δ421-454	19.7 ± 5.2	65.2 ± 5.4	partial +/-
	R423D	83.8 ± 2.0	75.9 ± 3.0	partial +/-
	K424A	85.4 ± 2.8	76.6 ± 0.8	+
	K468A K487A	20.8 ± 9.2	0.0 ± 0.0	-
	K468E K487D	35.0 ± 0.9	3.3 ± 3.3	-
	K468A K487A E567A D574A	6.1 ± 2.6	4.3 ± 4.3	-
	K468E K487D E567K D574K	16.6 ± 9.3	10.6 ± 6.0	-
	Y477A	42.2 ± 8.7	48.4 ± 2.6	+
	Y477A R712A	28.9 ± 3.5	0.0 ± 0.0	-
	D528K	1.0 ± 0.6	1.3 ± 0.8	-
	N539D K863D	4.8 ± 2.8	0.3 ± 0.2	-
	I541D	0.0 ± 0.0	2.9 ± 2.9	-
	D542K	0.5 ± 0.5	0.0 ± 0.0	-
	G547D	62.0 ± 5.9	55.8 ± 4.3	+
	K549A	19.3 ± 2.8	5.6 ± 4.9	-
	K549A E855A	62.0 ± 4.6	32.5 ± 4.8	+
	F555A	79.5 ± 2.5	73.3 ± 2.8	+
	L560A	76.1 ± 3.7	79.0 ± 4.0	+
	Δ561-588	0.0 ± 0.0	0.0 ± 0.0	-
	K561D	81.2 ± 1.2	76.1 ± 5.4	+
	R565D	79.9 ± 4.6	75.5 ± 4.7	+
	E567A D574A	17.5 ± 9.1	0.0 ± 0.0	-
	E567K D574K	42.6 ± 0.5	28.7 ± 4.2	partial +/-
	K571D	77.2 ± 6.5	76.5 ± 1.5	+
	D574K	76.2 ± 3.9	69.4 ± 3.5	partial +/-
	T578D	77.0 ± 7.6	37.3 ± 7.4	+
	R579K	80.9 ± 6.2	76.2 ± 7.4	partial +/-
	Y580D	32.0 ± 8.3	19.4 ± 3.0	+
	W581A	80.6 ± 2.0	70.2 ± 13.5	patial +/-
	SSS590-92AAA	71.8 ± 3.9	74.3 ± 1.3	+
	D597K	54.5 ± 7.3	55.0 ± 7.9	+

	Y602A	87.3 ± 2.2	79.4 ± 3.4	+
	W603A	69.4 ± 3.9	56.0 ± 11.8	+
	GIGR618AAAA	0.5 ± 0.5	0.0 ± 0.0	-
	L635S	77.9 ± 4.8	34.0 ± 1.5	+
	E640K	79.6 ± 2.9	47.6 ± 7.6	+
	D652K	77.2 ± 3.3	47.8 ± 6.1	+
	L653D	48.9 ± 5.8	39.9 ± 1.4	+
	K665E	72.7 ± 5.3	54.1 ± 4.8	+
	D671G	92.4 ± 2.7	61.1 ± 2.3	-
	D671A	73.1 ± 1.1	55.4 ± 10.2	-
	D671K	19.6 ± 5.3	9.9 ± 5.9	-
	E674G	10.3 ± 2.7	8.9 ± 0.7	partial +/-
	E674K	1.3 ± 1.0	0.9 ± 0.5	-
	K678A	61.8 ± 3.8	38.7 ± 1.9	partial +/-
	K678E	3.7 ± 1.2	0.0 ± 0.0	-
	P679A	74.3 ± 4.7	50.2 ± 4.4	+
	Y682A	72.9 ± 4.8	69.9 ± 5.7	+
	D685K	67.1 ± 4.5	77.9 ± 4.7	partial +/-
	D690K	83.3 ± 6.4	73.0 ± 2.8	+
	W691A	75.1 ± 2.1	77.5 ± 7.7	+
	R695Q	79.6 ± 3.2	54.0 ± 4.0	+
	C709S	85.4 ± 1.2	46.0 ± 7.8	+
	N710L	83.9 ± 3.6	44.4 ± 1.2	+
	R712A	25.5 ± 13.4	0.2 ± 0.2	-
	V720A	81.2 ± 10.0	83.8 ± 4.6	+
	E751A	36.2 ± 1.3	45.9 ± 5.1	+
	VL765AA	78.6 ± 1.3	83.6 ± 3.6	+
	K774A	76.2 ± 5.0	50.9 ± 2.1	+
	C777A	78.4 ± 3.7	78.1 ± 3.0	+
	K783E	89.1 ± 3.4	79.9 ± 3.2	+
	L791A	78.7 ± 3.2	75.0 ± 3.4	+
	VL803AA	81.5 ± 0.9	75.2 ± 4.9	+
	E809A	9.7 ± 3.1	3.4 ± 4.1	-
	F814D	1.4 ± 1.4	0.0 ± 0.0	-
	Y816A	1.9 ± 1.1	0.0 ± 0.0	-
	I820A	48.5 ± 3.8	42.4 ± 9.7	partial +/-
	E821K	78.7 ± 7.2	76.1 ± 4.6	+
	L826D	0.9 ± 0.9	0.3 ± 0.3	-
	L829D	5.4 ± 2.9	2.3 ± 1.6	-
	Δ849-868	3.4 ± 2.2	0.0 ± 0.0	-
	R853A	0.0 ± 0.0	1.2 ± 1.2	partial +/-
	E855A	17.5 ± 1.2	4.0 ± 4.9	-