Table S1. Details about all simulated systems. Systems for which the uniprot ID is provided means that the available AlphaFold (AF) model was used. All oligomers were predicted with AF multimer. The number of chains in each system is in parentheses. Total events are computed after removing the first 2 microseconds (equilibration) in each replica.

ID	System (number of chains)	Organism	Source (Uniprot ID, PDB code or AlphaFold)	Total # of DOPC lipids	Total events	MD run length
1	TMEM16K (2)	*H. sapiens*	PDB: 5OC9	1000	251	20 µs
2	TMEM16K (2)	*H. sapiens*	PDB: 6R65	1000	278	20 µs
3	TMEM16K (2)	*H. sapiens*	PDB: 6R7X	1000	10	20 µs
4	TMEM16K (2)	*H. sapiens*	PDB: 6R7Y	1000	7	20 µs
5	TMEM16K (2)	*H. sapiens*	PDB: 6R7Z	1000	28	20 µs
6	TMEM16F (2)	*M. musculus*	PDB: 8B8Q	800	27	20 µs
7	TMEM16A (2)	*M. musculus*	PDB: 7ZK3	800	29	20 µs
8	TMEM41B (1)	*H. sapiens*	ID: Q5BJD5	600	22	20 µs
9	VMP1 (1)	*H. sapiens*	ID: Q96GC9	600	24	20 µs
10	TMEM41B - VMP1 (2)	*H. sapiens*	ID: Q5BJD5 ID: Q96GC9	800	31	20 µs
11	ATG9 (3)	*S. pombe*	PDB: 7D0I	1200	175	20 µs
12	ATG9A (3)	*H. sapiens*	PDB: 6WQZ	1200	93	20 µs
13	VDAC1 (1)	*H. sapiens*	PDB: 6G6U	600	24	20 µs
14	VDAC1 (2)	*H. sapiens*	PDB: 6G6U	800	161	20 µs
15	VDAC2 (1)	*D. rerio*	PDB: 4BUM	800	25	20 µs
16	VDAC2 (2)	*D. rerio*	PDB: 4BUM	800	231	20 µs
17	VDAC3 (1)	*H. sapiens*	ID: Q9Y277	800	24	20 µs
18	VDAC3 (2)	*H. sapiens*	ID: Q9Y277	800	319	20 µs
19	Rhodopsin (1)	*B. taurus*	PDB: 4A4M	800	19	20 µs
20	Rhodopsin (2)	*B. taurus*	PDB: 4A4M	800	39	20 µs
21	MCP1 (1)	*S. cerevisiae*	ID: Q12106	600	26	20 µs
22	MCP1 (2)	*S. cerevisiae*	ID: Q12106	800	33	20 µs
23	Xk (1)	*H. sapiens*	ID: P51811	800	9	20 µs
24	Xkr8 (1)	*H. sapiens*	PDB: 7DCE	800	11	20 µs

25	Xkr9 (1)	*R. norvegicus*	PDB: 7P16	800	8	20 µs
26	GlpG (1)	*E. colii*	PDB: 3B45	600	3	20 µs
27	Spns1 (1)	*H. sapiens*	ID: Q9H2V7	800	3	20 µs
28	MFSD2A (1)	*H. sapiens*	PDB: 7OIX	800	5	20 µs
29	ABCB1 (1)	*H. sapiens*	PDB: 7A69	1000	4	20 µs
30	ABCB4 (4)	*H. sapiens*	PDB: 6S7P	800	9	20 µs
31	ABCB11 (11)	*H. sapiens*	PDB: 6LR0	800	8	20 µs
32	ABCG40 (1)	*A. thaliana*	ID: Q9M9E1	1200	2	20 µs
33	MsbA (2)	*E. colii*	PDB: 7PH4	800	1	20 µs
34	PglK (2)	*C. jejuni*	PDB: 5C73	800	15	20 µs
35	PglK (2)	*C. jejuni*	PDB: 5C76	1000	18	20 µs
36	PglK (2)	*C. jejuni*	PDB: 5C78	1000	19	20 µs
37	SERCA2b (1)	*H. sapiens*	PDB: 7E7S	800	9	20 µs
38	DGGGP (1)	*M. jannaschii*	PDB: 6M31	800	1	20 µs
39	DGGGP (2)	*M. jannaschii*	PDB: 6M31	800	1	20 µs
40	Drs2p (1)	*S. cerevisiae*	PDB: 6ROH	1200	8	20 µs
41	Drs2p (1)	*S. cerevisiae*	PDB: 6ROI	1200	7	20 µs
42	Drs2p (1)	*S. cerevisiae*	PDB: 6ROJ	1000	3	20 µs
43	VAMP2 (1)	*H. sapiens*	ID: P63027	600	0	20 µs
44	VAMP2 (2)	*H. sapiens*	ID: P63027	1000	2	20 µs
45	YidC (1)	*B. halodurans*	PDB: 3WO6	600	16	20 µs
46	YidC R72A (1)	*B. halodurans*	PDB: 3WO6	800	8	20 µs
47	YidC (1)	*B. halodurans*	PDB: 3WO7	800	10	20 µs
48	MisCB (1)	*B. subtilis*	ID: P54544	800	33	20 µs
49	Oxa1 (1)	*S. cerevisiae*	ID: P39952	800	45	20 µs
50	OXA1L (1)	*H. sapiens*	PDB: 6ZM5	800	18	20 µs
51	OXA1L-peptide (2)	*H. sapiens*	PDB: 6ZM5	800	0	20 µs
52	Cox18 (1)	*S. cerevisiae*	ID: P53239	800	47	20 µs

53	Alb3 (1)	*A. thaliana*	ID: Q8LBP4	800	24	20 µs
54	Emc1 (1)	*S. cerevisiae*	PDB: 6WB9	800	0	20 µs
55	Emc3 (1)	*S. cerevisiae*	PDB: 6WB9	800	113	20 µs
56	Emc4 (1)	*S. cerevisiae*	PDB: 6WB9	800	115	20 µs
57	Emc5 (1)	*S. cerevisiae*	PDB: 6WB9	800	0	20 µs
58	Emc6 (1)	*S. cerevisiae*	PDB: 6WB9	800	1	20 µs
59	EMC complex (8)	*S. cerevisiae*	PDB: 6WB9	1000	170	20 µs
60	Get1 (1)	*S. cerevisiae*	ID: P53192	800	56	20 µs
61	Get1 (1) F113L W147L W189L	*S. cerevisiae*	ID: P53192	600	85	20 µs
62	Get1 (1) F113L G120L W147L G185L W189L	*S. cerevisiae*	ID: P53192	600	22	20 µs
63	Get1 (1) K16L Q19L R104L K116L W189L N193L N196L Q199L	*S. cerevisiae*	ID: P53192	800	36	20 µs
64	Get1 (1) T109L K116L K121L W147L Y149L S183L G185L W189L N193L N196L	*S. cerevisiae*	ID: P53192	800	0	20 µs
65	Get2 (1)	*S. cerevisiae*	ID: P40056	800	7	20 µs
66	Get1 - Get2 (2)	*S. cerevisiae*	ID: P53192 ID: P40056	800	71	20 µs
67	GET complex (4)	*S. cerevisiae*	ID: P53192 ID: P40056	800	142	20 µs
68	GET complex (4) R148L K328L R416L K428 K433L E511L	*S. cerevisiae*	ID: P53192 ID: P40056	800	176	20 µs
69	GET complex (4) T421L K428L K433L W459L Y461L S495L G497L W501L N505L N508L	*S. cerevisiae*	ID: P53192 ID: P40056	800	22	20 µs
70	GET complex (4) T421L K428L G432L K433L G458L W459L Y461L S495L G497L W501L N505L N508L	*S. cerevisiae*	ID: P53192 ID: P40056	800	22	20 µs
71	WRB (1)	*H. sapiens*	PDB: 6SO5	800	7	20 µs

72	CAML (1)	*H. sapiens*	PDB: 6SO5	800	2	20 µs
73	WRB - CAML (2)	*H. sapiens*	PDB: 6SO5	800	22	20 µs
74	WRB - CAML (4)	*H. sapiens*	PDB: 6SO5	800	55	20 µs
75	TRC complex (6)	*H. sapiens*	PDB: 6SO5	800	39	20 µs
76	TMCO1 (1)	*H. sapiens*	PDB: 6W6L	600	70	20 µs
77	C20orf24 (1)	*H. sapiens*	PDB: 6W6L	600	14	20 µs
78	GEL complex (2)	*H. sapiens*	PDB: 6W6L	800	80	20 µs
79	Nicalin (1)	*H. sapiens*	PDB: 6W6L	600	0	20 µs
80	NOMO (1)	*H. sapiens*	PDB: 6W6L	600	0	20 µs
81	TMEM147 (1)	*H. sapiens*	PDB: 6W6L	600	12	20 µs
82	BOS complex (3)	*H. sapiens*	PDB: 6W6L	800	19	20 µs
83	PAT complex (2)	*H. sapiens*	ID: Q9Y284 PDB: 6W6L	800	5	20 µs
84	Asterix (1)	H. sapiens	ID: Q9Y284	600	121	20 µs
85	Asterix (1) M37L N38L M42L S45L M46L M50L Q79L M80L M81L S82L S83L M85L S89L M93L S94L	H. sapiens	ID: Q9Y284	800	27	20 µs
86	CCDC47 (1)	H. sapiens	PDB: 6W6L	1000	2	20 µs
87	Atp13a1 (1)	*H. sapiens*	ID: Q9HD20	800	26	20 µs
88	Atp13a1 (1)	*M. musculus*	ID: Q9EPE9	800	20	20 µs
89	Shr3 (1)	*S. cerevisiae*	ID: Q02774	800	3	20 µs
90	Hrd1 (1)	*S. cerevisiae*	PDB: 6VJZ	800	22	20 µs
91	Der1 (1)	*S. cerevisiae*	PDB: 6VJZ	800	1	20 µs
92	Hrd1-Der1 (2)	*S. cerevisiae*	PDB: 6VJZ	800	28	20 µs
93	ERAD complex (4)	*S. cerevisiae*	PDB: 6VJZ	800	32	20 µs
94	Sec61 α - Sec61 γ (2)	*C. familiaris*	PDB: 6Z3T	800	98	20 µs
95	SEC61 complex (3)	*H. sapiens*	PDB: 8DNV	800	26	20 µs
96	SEC61 complex (3)	*H. sapiens*	PDB: 8DNW	800	42	20 µs
97	Sec61 α (1)	*H. sapiens*	PDB: 8B6L	800	46	20 µs

98	Sec61 β (1)	*H. sapiens*	PDB: 8B6L	800	0	20 µs
99	Sec61 γ (1)	*H. sapiens*	PDB: 8B6L	800	0	20 µs
100	SEC61 complex (3)	*H. sapiens*	PDB: 8B6L	800	13	20 µs
101	Trap α (1)	*H. sapiens*	PDB: 8B6L	800	0	20 µs
102	Trap β (1)	*H. sapiens*	PDB: 8B6L	800	0	20 µs
103	Trap γ (1)	*H. sapiens*	PDB: 8B6L	800	20	20 µs
104	Trap δ (1)	*H. sapiens*	PDB: 8B6L	800	0	20 µs
105	Trap β - Trap δ - Trap γ (3)	*H. sapiens*	PDB: 8B6L	800	4	20 µs
106	TRAP complex (4)	*H. sapiens*	PDB: 8B6L	800	7	20 µs
107	SEC61 complex - TRAP complex (7)	*H. sapiens*	PDB: 8B6L	1000	26	20 µs
108	RPN1 (1)	*H. sapiens*	PDB: 8B6L	800	0	20 µs
109	RPN2 (1)	*H. sapiens*	PDB: 8B6L	1200	0	20 µs
110	OST4 (1)	*H. sapiens*	PDB: 8B6L	600	1	20 µs
111	OST48 (1)	*H. sapiens*	PDB: 8B6L	800	1	20 µs
112	DAD1 (1)	*H. sapiens*	PDB: 8B6L	600	32	20 µs
113	STT3a (1)	*H. sapiens*	PDB: 8B6L	800	50	20 µs
114	TMEM258 (1)	*H. sapiens*	PDB: 8B6L	600	3	20 µs
115	OSTC (1)	*H. sapiens*	PDB: 8B6L	800	132	20 µs
116	OSTA complex (8)	*H. sapiens*	PDB: 8B6L	1600	35	20 µs
117	SEC61 complex - OSTA complex (11)	*H. sapiens*	PDB: 8B6L	1600	35	20 µs
118	SEC61 complex - TRAP complex - OSTA complex (15)	*H. sapiens*	PDB: 8B6L	1800	72	20 µs
119	Tom5 (1)	*S. cerevisiae*	PDB: 6UCU	800	0	20 µs
120	Tom6 (1)	*S. cerevisiae*	PDB: 6UCU	800	0	20 µs
121	Tom7 (1)	*S. cerevisiae*	PDB: 6UCU	800	0	20 µs
122	Tom22 (1)	*S. cerevisiae*	PDB: 6UCU	800	0	20 µs
123	Tom40 (1)	*S. cerevisiae*	PDB: 6UCU	800	30	20 µs
124	TOM complex (5)	*S. cerevisiae*	PDB: 6UCU	800	56	20 µs

125	TOM complex (10)	*S. cerevisiae*	PDB: 6UCU	1200	119	20 µs
126	BamA (1)	*E. colii*	PDB: 6QGW	800	30	20 µs
127	TamA (1)	*E. colii*	PDB: 4C00	800	112	20 µs
128	Oep80 (1)	*A. thaliana*	ID: Q9C5J8	800	26	20 µs
129	Sam50 (1)	*S. cerevisiae*	PDB: 7BTW	600	125	20 µs
130	Sam50 (1)	*S. cerevisiae*	PDB: 7BTX	800	84	20 µs
131	Sam50 - Sam50 (2)	*S. cerevisiae*	PDB: 7BTW	800	76	20 µs
132	Sam50 - Mdm10 (2)	*S. cerevisiae*	PDB: 7BTY	800	54	20 µs
133	Sam50 - Tom40 (2)	*S. cerevisiae*	PDB: 7E4H	800	77	20 µs
134	SAM complex (3)	*S. cerevisiae*	PDB: 7BTX	800	54	20 µs
135	Tim22 (1)	*S. cerevisiae*	PDB: 6LO8	800	127	20 µs
136	Tim18 (1)	*S. cerevisiae*	PDB: 6LO8	800	56	20 µs
137	Tim54 (1)	*S. cerevisiae*	PDB: 6LO8	800	5	20 µs
138	Sdh3 (1)	*S. cerevisiae*	PDB: 6LO8	800	15	20 µs
139	TIM22 complex (10)	*S. cerevisiae*	PDB: 6LO8	1000	246	20 µs
140	Tim22 (1)	*H. sapiens*	PDB: 7CGP	800	49	20 µs
141	TIM22 complex (15)	*H. sapiens*	PDB: 7CGP	1200	60	20 µs
142	Tim23 (1)	*S. cerevisiae*	PDB: 8SCX	800	2	20 µs
143	Tim17 (1)	*S. cerevisiae*	PDB: 8SCX	800	250	20 µs
144	Tim17 D17L, D76L, E126L (1)	*S. cerevisiae*	PDB: 8SCX	800	127	20 µs
145	Mgr2 (1)	*S. cerevisiae*	ID: Q02889	800	27	20 µs
146	Tim17 - Mgr2 (2)	*S. cerevisiae*	PDB: 8SCX ID: Q02889	800	7	20 µs
147	Tim17 - Mgr2 - Tim23 (3)	*S. cerevisiae*	PDB: 8SCX ID: Q02889	800	29	20 µs
148	Tim17 - Tim23 - Tim44 - Mgr2 (4)	*S. cerevisiae*	PDB: 8SCX ID: Q02889	800	21	20 µs
149	TIM23 complex (3)	*S. cerevisiae*	PDB: 8SCX	1000	157	20 µs
150	Tim17 (1)	*H. sapiens*	ID: Q99595	800	213	20 µs
151	Tim17	*H. sapiens*	ID: Q99595	800	124	20 µs

	D16L, D77L, E127L (1)					
152	Tim23 (1)	*H. sapiens*	ID: O14925	800	30	20 µs
153	Tim17 - Tim23 (2)	*H. sapiens*	ID: Q99595 ID: O14925	800	182	20 µs
154	Tim50 (1)	*H. sapiens*	ID: Q3ZCQ8	1200	0	20 µs
155	TIM23 complex (3)	*H. sapiens*	ID: Q99595 ID: O14925 ID: Q3ZCQ8	1000	170	20 µs
156	MTCH1 (1)	*H. sapiens*	ID: Q9NZJ7	600	129	20 µs
157	MTCH2 (1)	*H. sapiens*	ID: Q9Y6C9	800	126	20 µs
158	MTCH2 Q29L, H142L, D189L, N242L (1)	*H. sapiens*	ID: Q9Y6C9	800	93	20 µs
159	MTCH2-peptide (2)	*H. sapiens*	ID: Q9Y6C9	800	2	20 µs
160	SLC25A46 (1)	*H. sapiens*	ID: Q96AG3	800	22	20 µs
161	SLC26A9 (2)	*H. sapiens*	PDB: 7CH1	1000	35	20 µs
162	SLC25A17 (1)	*H. sapiens*	ID: O43808	800	20	20 µs

Supporting Figures

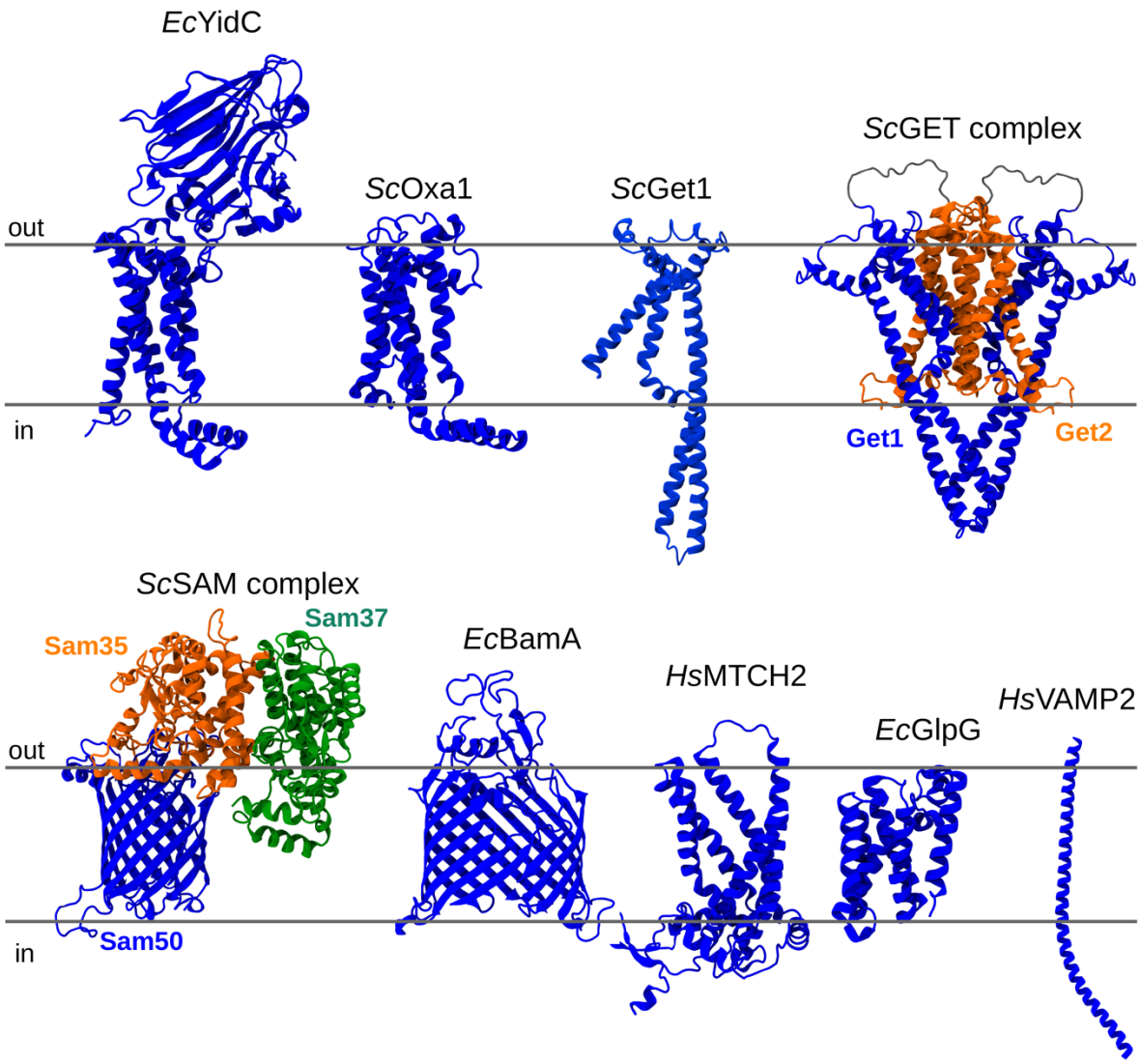

Figure S1. 3D structure of experimentally tested proteins.

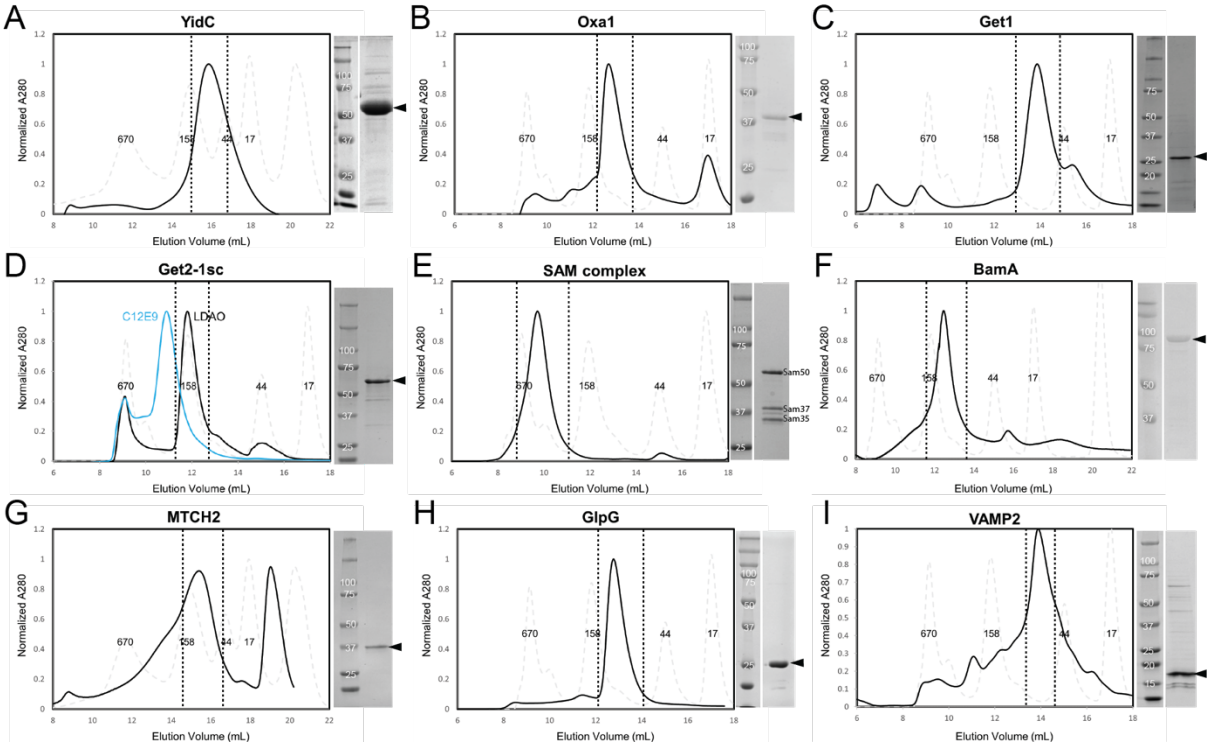

Figure S2. Size-exclusion chromatography analyses of proteins used in reconstitutions. Proteins for *in vitro* reconstitution were purified by affinity followed by size exclusion chromatography. Traces are shown (black) along with traces of the molecular weight standards (dotted grey); fractions used for reconstitutions are indicated (dotted black lines). Note that the elution volume of each protein reflects its size including detergent micelle. SDS-PAGE analysis of the purified protein is to the right of each chromatographic trace.

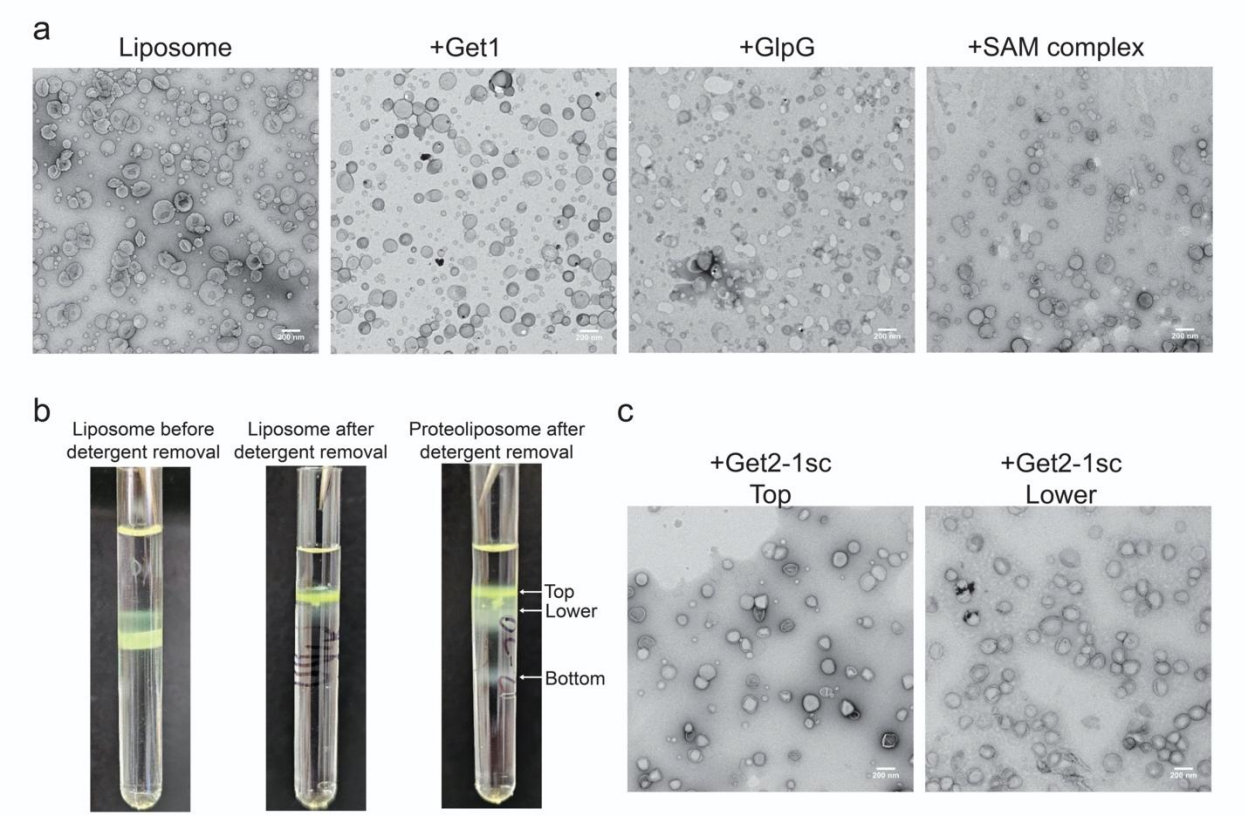

Figure S3. Liposome characterization by negative staining TEM and liposome floatation. (a) Examples of floated liposomes and proteoliposomes used for the scramblase assay as assessed by negative stain TEM. The preparations comprise both ~200nm diameter liposomes formed by extrusion as well as tiny liposomes remaining from the rehydration process (<50nm). (b) Detergent-treated liposomes, where detergent is not removed with Biobeads, likely have lesions that allow them to absorb Optiprep solution, making them sink in the density gradient (left panel). Same liposomes treated with Biobeads to remove detergent, and so presumably intact, float at the top (middle panel). Proteoliposomes used in our assays float near the top (right panel: layers labeled “top” and “lower”); a “bottom” fraction most likely has lesions and was (discarded). Proteoliposomes are expected to be heavier than protein-free liposomes and to concentrate in the fraction labelled “lower” versus the very “top”.(c) Negative stain analysis of the proteoliposomes in the very “top” layer (in b, right) shows a mixture of 200nm and <50nm liposomes, whereas the “lower” fraction (in b, right) only has 200nm vesicles. The tiny liposomes likely are refractory to protein reconstitution due to their high curvature, and are expected to be lighter than proteoliposomes.

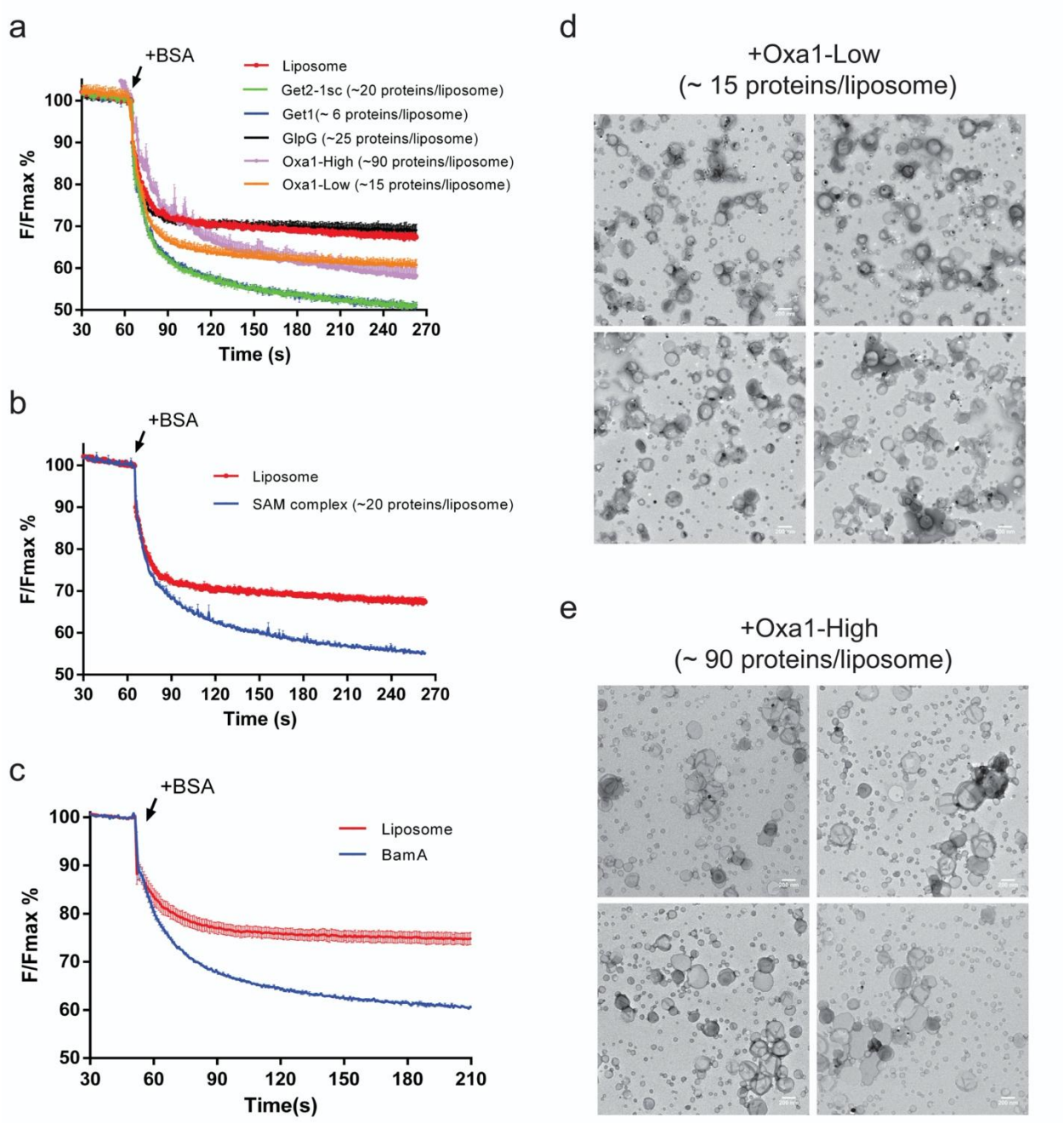

Figure S4. Scrambling activity measured by spectrofluorometer confirms the fast scrambling of Get1, Get2-1sc, the Sam50 complex, BamA and Oxa1 (at lower concentrations). (a) Get1, Get2-1sc, Oxa1 at lower protein concentration (~15 proteins/liposome), but not GlpG (~15 proteins/liposome), show fast scrambling. The traces of fluorescence decay show a similar t1/2 (~7s) for both protein-free liposomes and proteoliposomes, suggesting the rate-limiting step is BSA back-extraction and quenching rather than lipid scrambling. Oxa1 at higher protein concentration (~90 proteins/liposome) show slower fluorescence decay. (b,c) Similarly, BamA and the SAM50 complex show fast scrambling as assessed by spectrofluorometer. (d,e) As shown by negative-staining electron microscopy, the Oxa1 traces that appear to drop slowly in (a) and in Fig. 1c are likely the result of liposome clustering/aggregation, which hinders BSA access to the membrane and slows back-extraction.

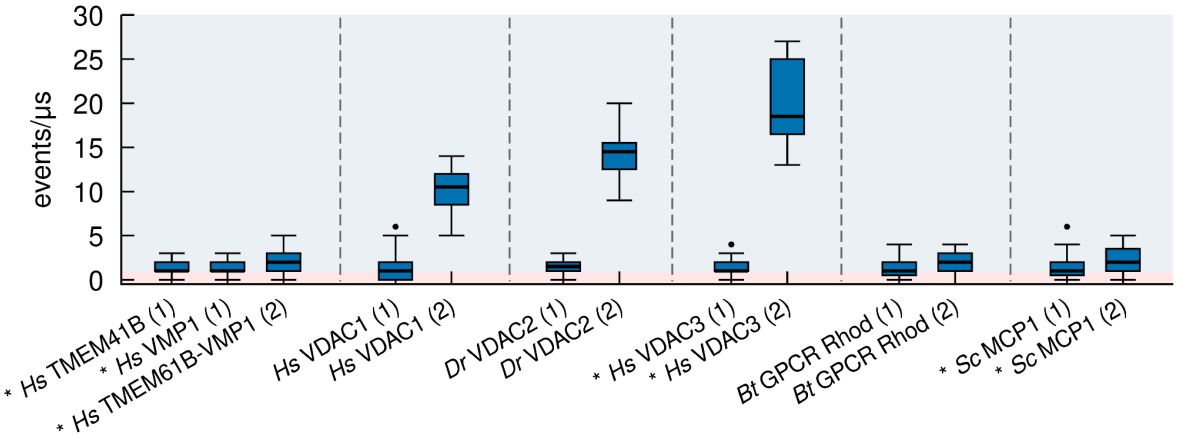

Figure S5. Oligomerization influences lipid scrambling. Comparison of lipid scrambling activity between monomeric and dimeric forms of selected scramblases. The blue shaded region represents the behavior for a scrambling-positive protein and the red area for a scrambling-negative protein. AlphaFold structures are denoted by the * symbol, oligomerization state is in parenthesis.

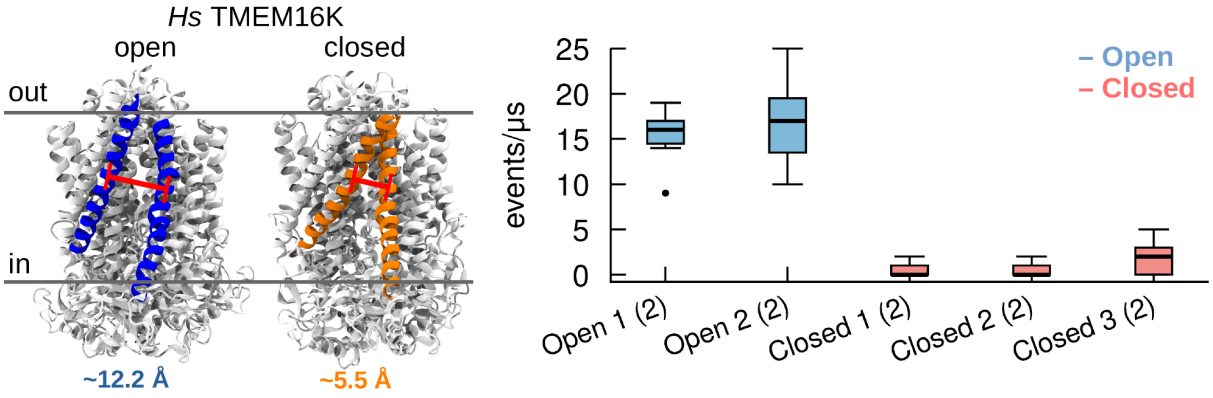

Figure S6. CG-MD simulations recapitulate conformational-dependent lipid scrambling activity by TMEM16K. Left: representative open and closed structures of TMEM16K. Right: Lipid scrambling events in different simulations of TMEM16K.

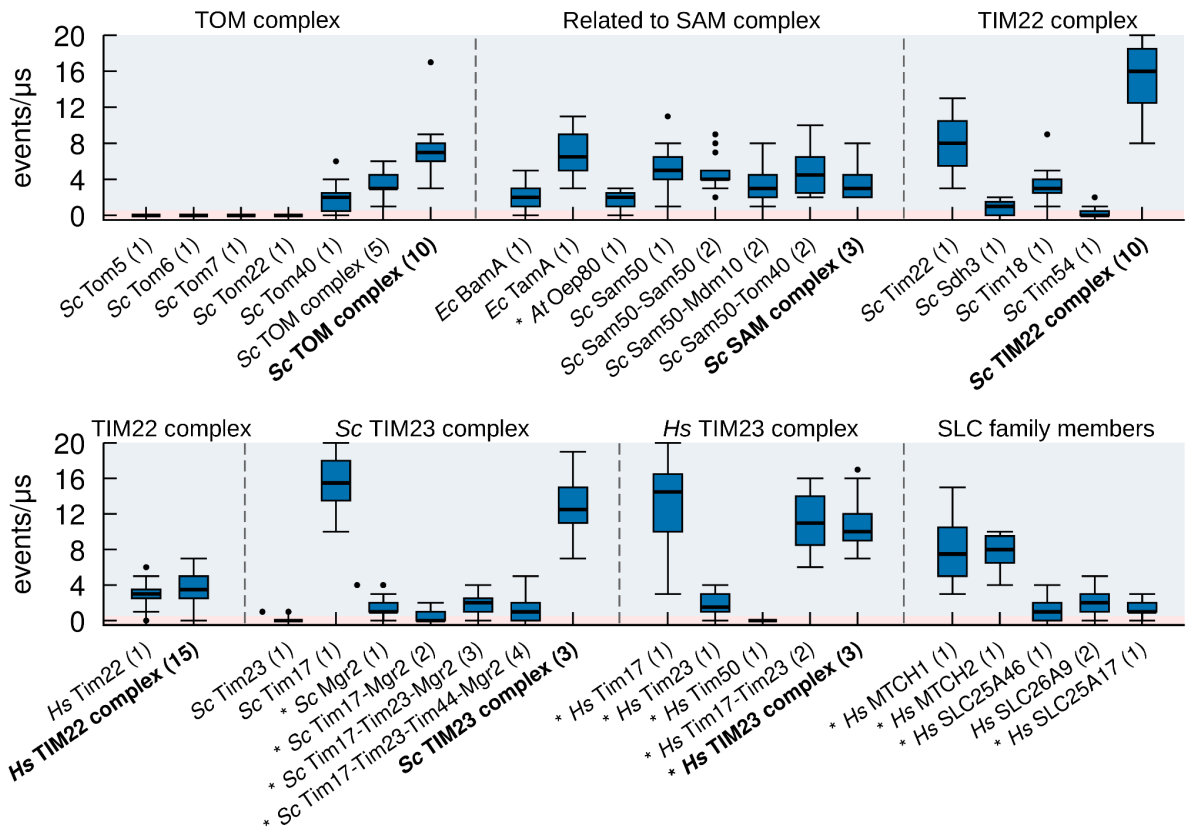

Figure S7. Extended version of insertase complexes in the mitochondria. Lipid scrambling activity of individual components and selected complexes for the major mitochondrial insertases investigated. The blue shaded region represents the behavior for a scrambling-positive protein and the red area for a scrambling-negative protein. AF structures are denoted by the * symbol, oligomerization state is in parenthesis.

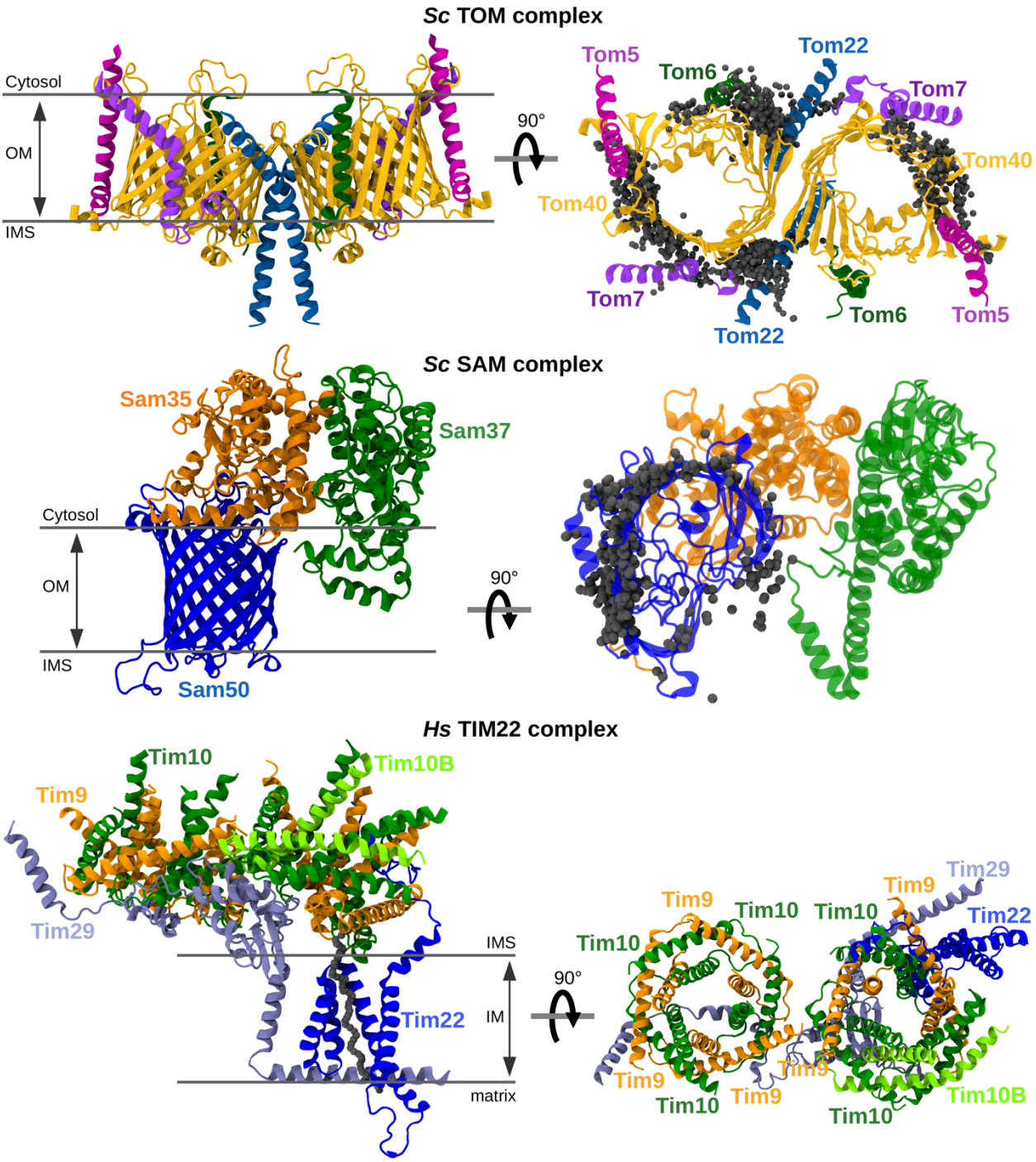

Figure S8. 3D structures of mitochondrial complexes TOM, SAM and *Hs*TIM22. The main lipid scrambling pathways are shown in gray beads. Since for the TOM and SAM complexes (mainly beta-barrels) more than one clearly path was observed, the path for all lipid scrambling events is shown. OM: outer membrane, IM: inner membrane, IMS: inter membrane space.

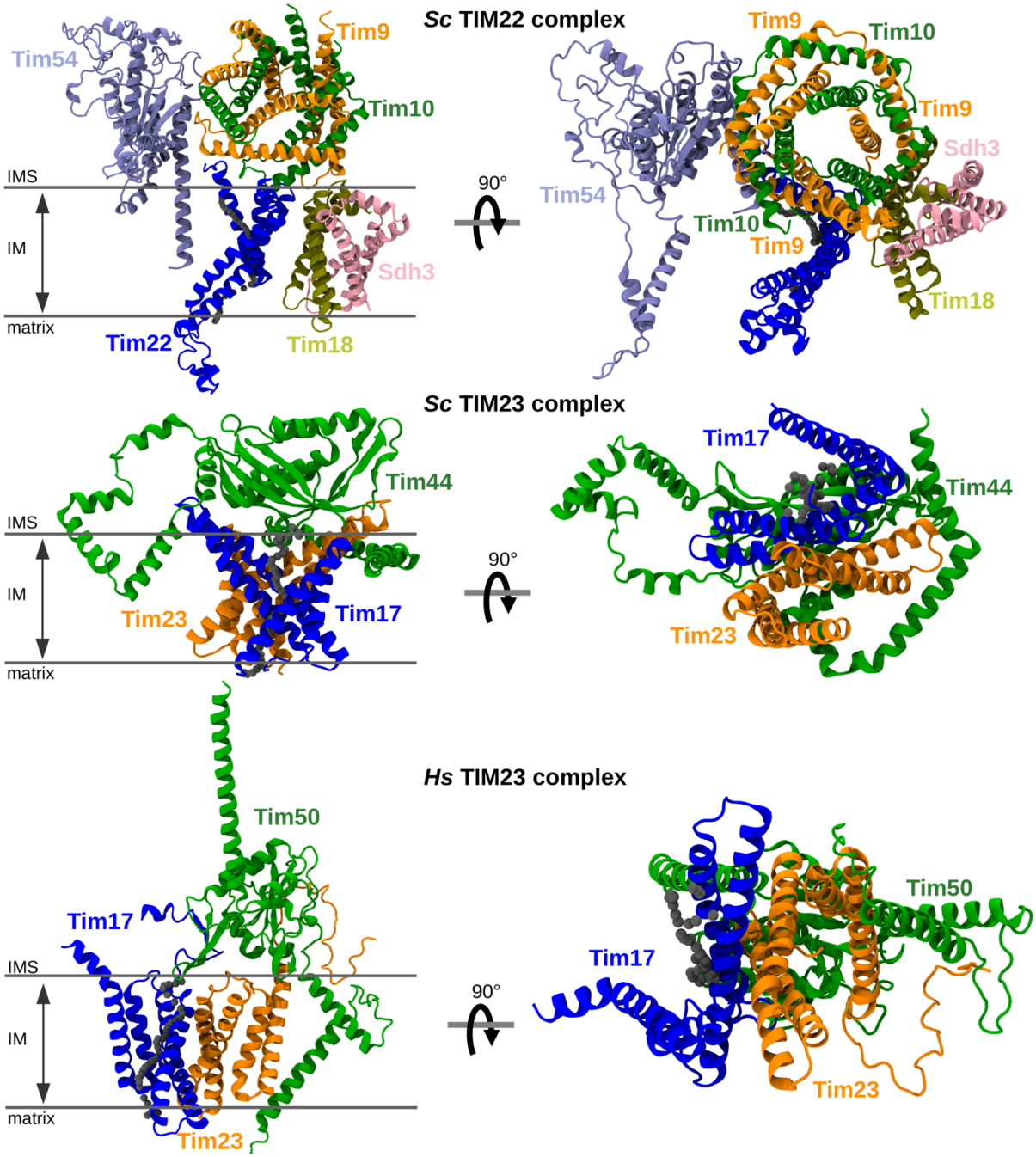

Figure S9. 3D structures of mitochondrial complexes *Sc*TIM22, *Sc*TIM23, *Hs*TIM23. The main lipid scrambling pathways are shown in gray beads. IM: inner membrane; IMS: inter membrane space.

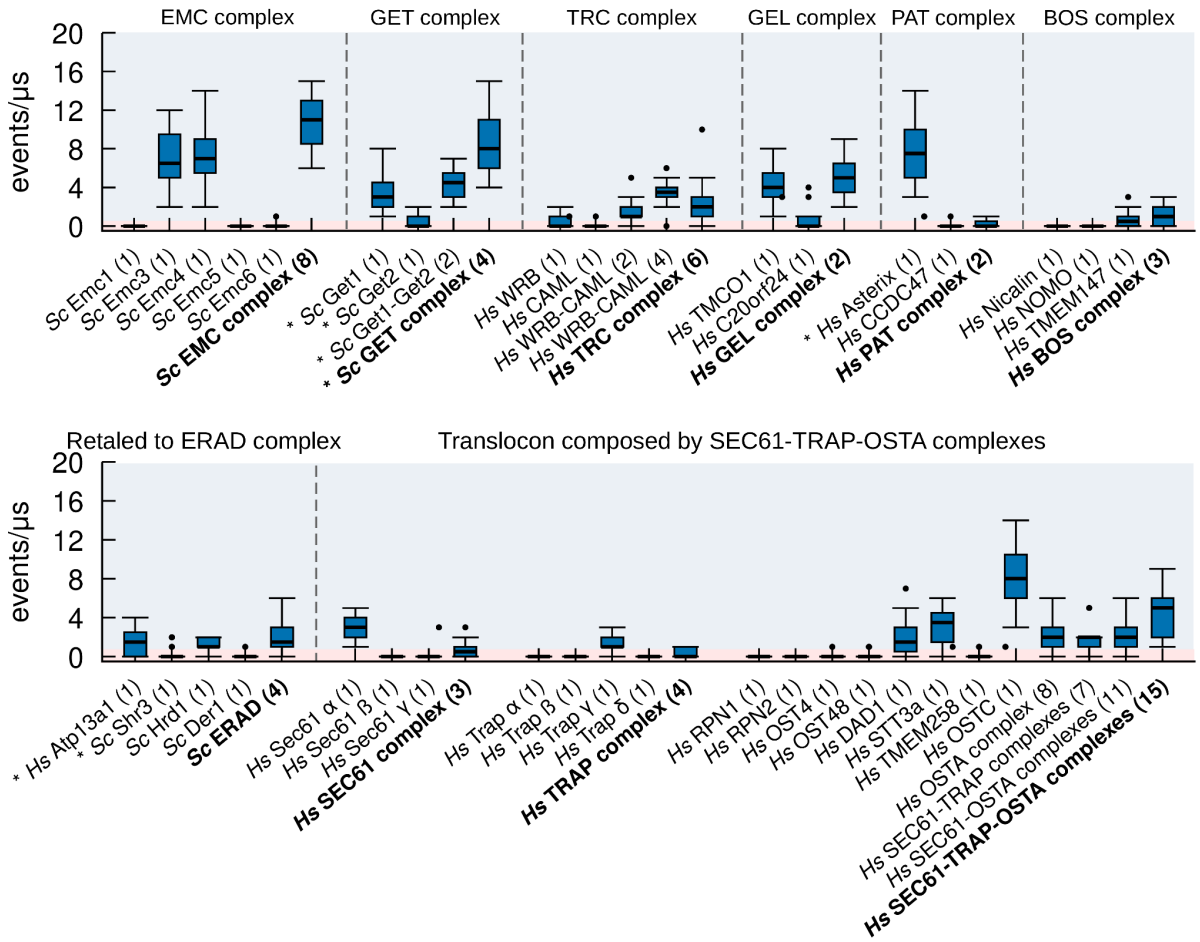

Figure S10. Extended version of insertase complexes in the endoplasmic reticulum. Lipid scrambling activity of individual components and selected complexes for the major ER insertases investigated. The blue shaded region represents the behavior for a scrambling-positive protein and the red area for a scrambling-negative protein. AlphaFold structures are denoted by the * symbol, oligomerization state is in parenthesis.

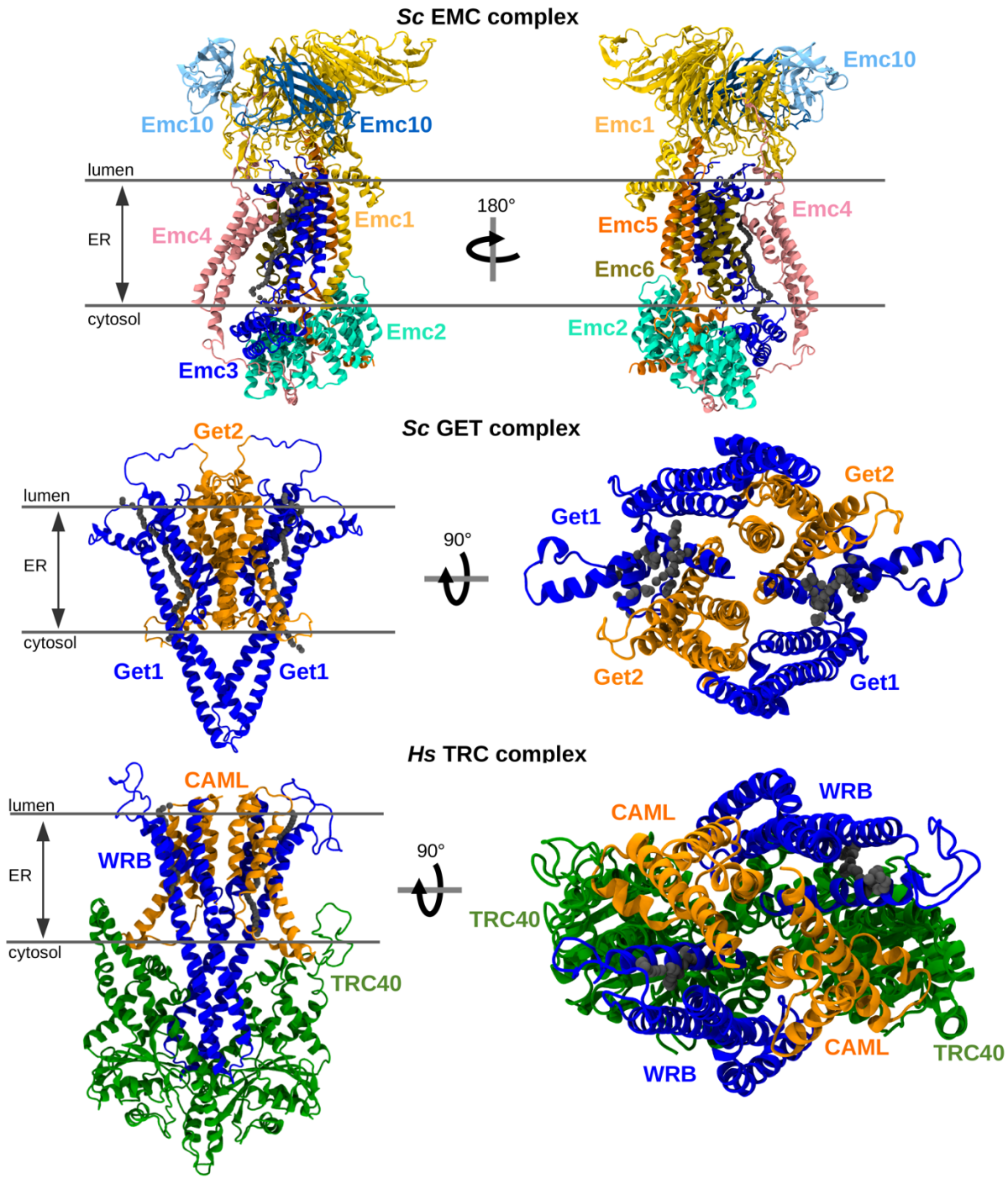

Figure S11. 3D structures of ER complexes EMC, GET and TRC. The main lipid scrambling pathways are shown in gray beads.

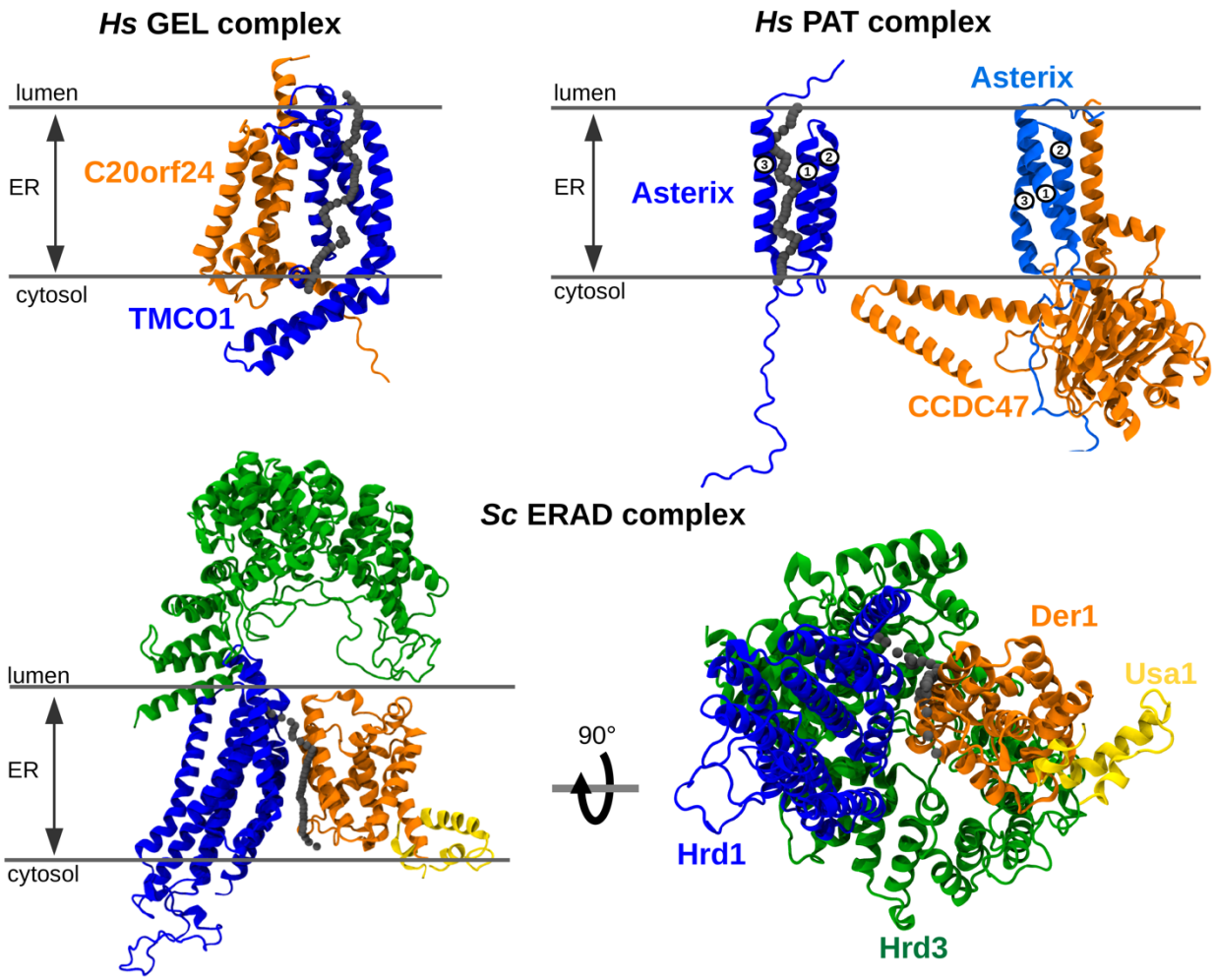

Figure S12. 3D structures of ER complexes GEL, PAT, ERAD. The main lipid scrambling pathways are shown in gray beads. Asterix's three helices are numbered to highlight the conformational change of Asterix following the presence of its partner CCDC47.

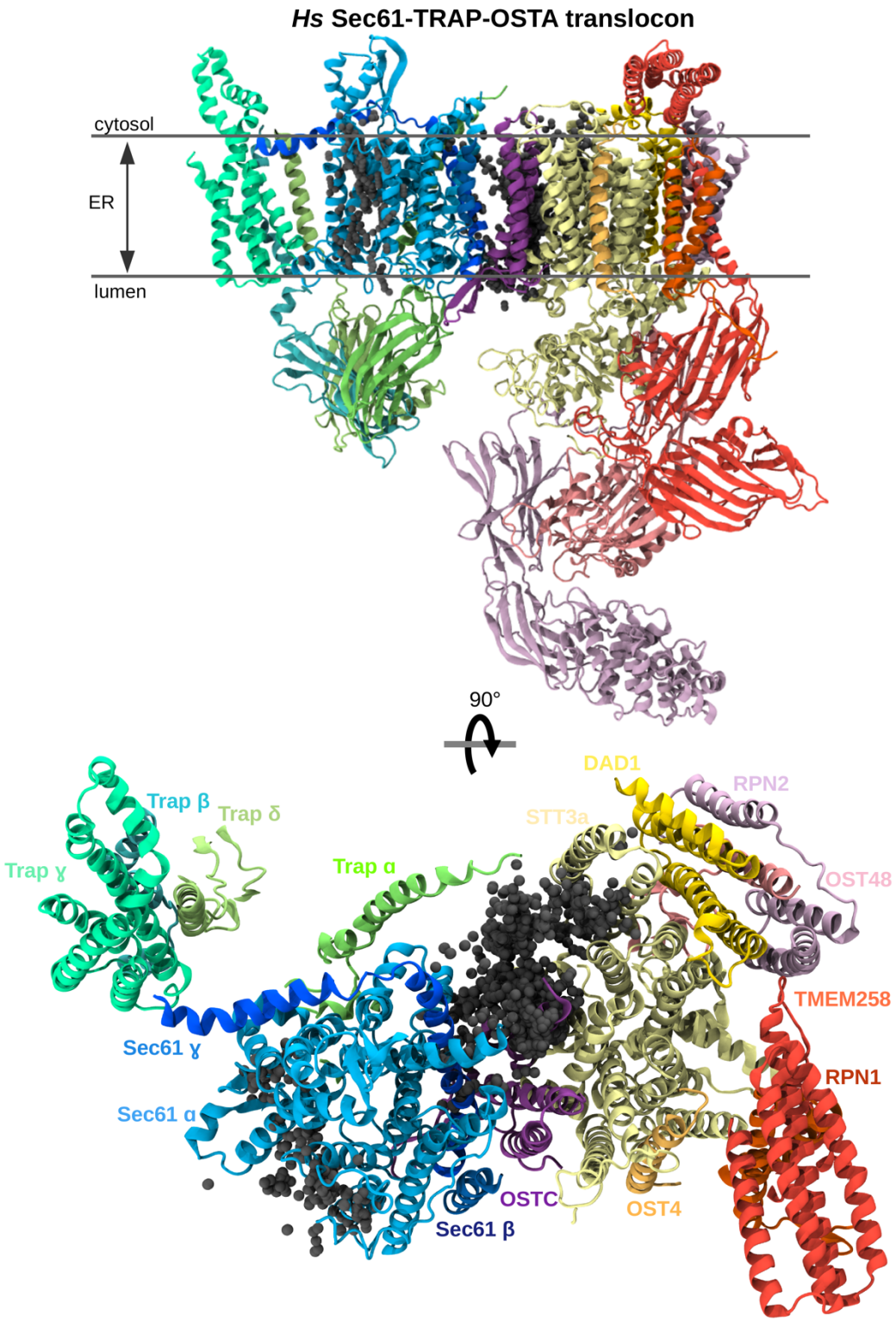

Figure S13. 3D structure of *Hs*SEC61-TRAP-OSTA complex. The lipid scrambling pathways for all the events obtained in one replica are shown in gray beads.

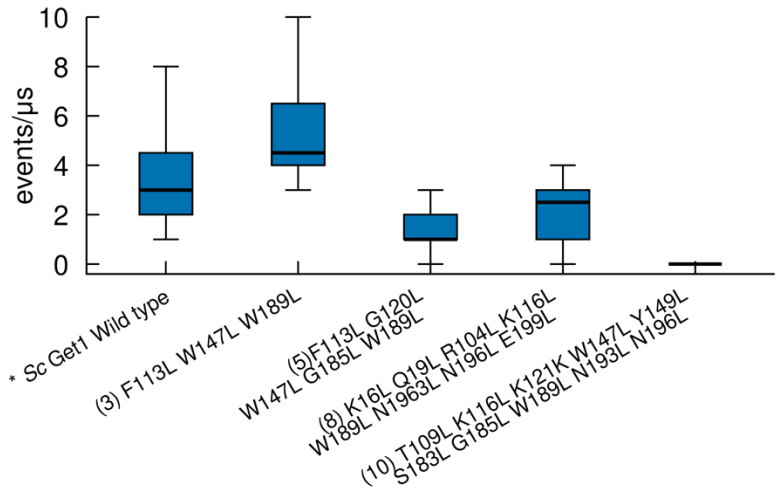

Figure S14. In silico mutational analysis of lipid scrambling in Get1. Scrambling activity calculations from CG-MD simulations of the wild type and various mutants of the ScGet1 subunit. 10 mutations (10L) are required to completely ablate lipid scrambling activity in ScGet1.