

Cardiolipin, and not monolysocardiolipin, preferentially binds to the interface of Complexes III and IV

Robin A. Corey, Noah Harrison, Phillip J. Stansfeld, Mark S.P. Sansom, Anna Duncan

Supplementary Data

1. Coordinates of CL and MLCL binding sites, as identified by PyLipID, for yeast, mouse and human supercomplexes (6 files)
2. Itp file for CL > MLCL used for FEP

Supplementary Figures

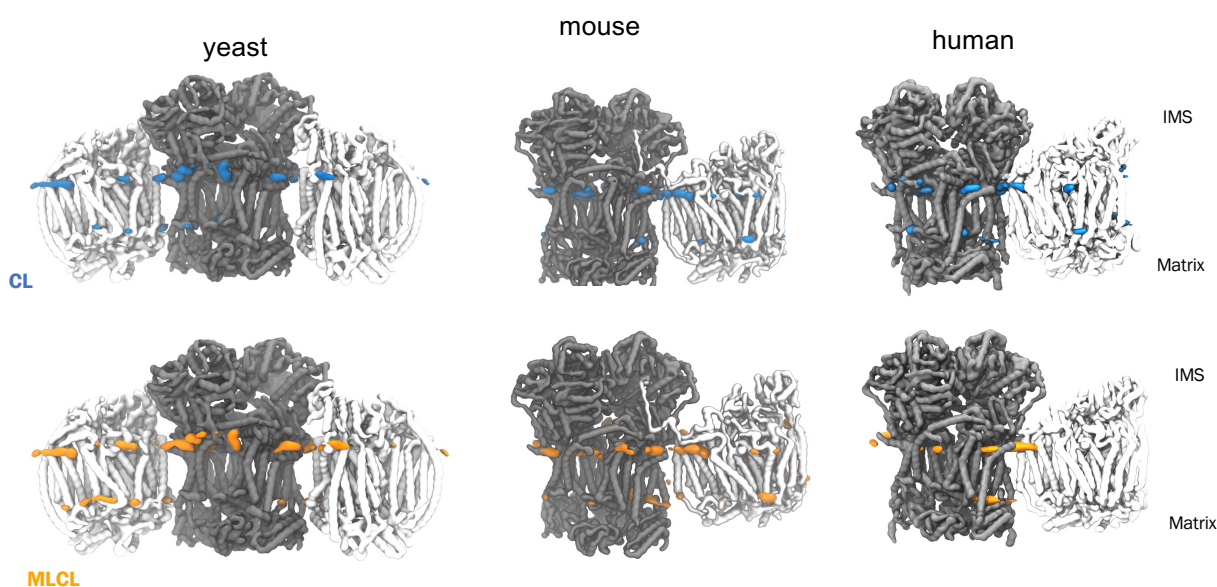
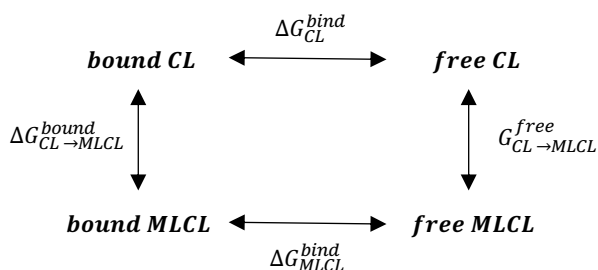


Figure S1. CL (top row) and MLCL (second row) headgroup density from yeast, mouse and human CIII₂CIV₁₋₂ CG MD simulations. CIII backbone is shown in grey and CIV in white, with CL headgroup densities in blue and MLCL densities in orange.



$$\begin{aligned}\Delta\Delta G &= \Delta G_{CL}^{bind} - \Delta G_{MLCL}^{bind} \\ &= \Delta G_{CL \rightarrow MLCL}^{bound} - \Delta G_{CL \rightarrow MLCL}^{free}\end{aligned}$$

Figure S2. Thermodynamic cycle used for the free energy perturbation simulations.

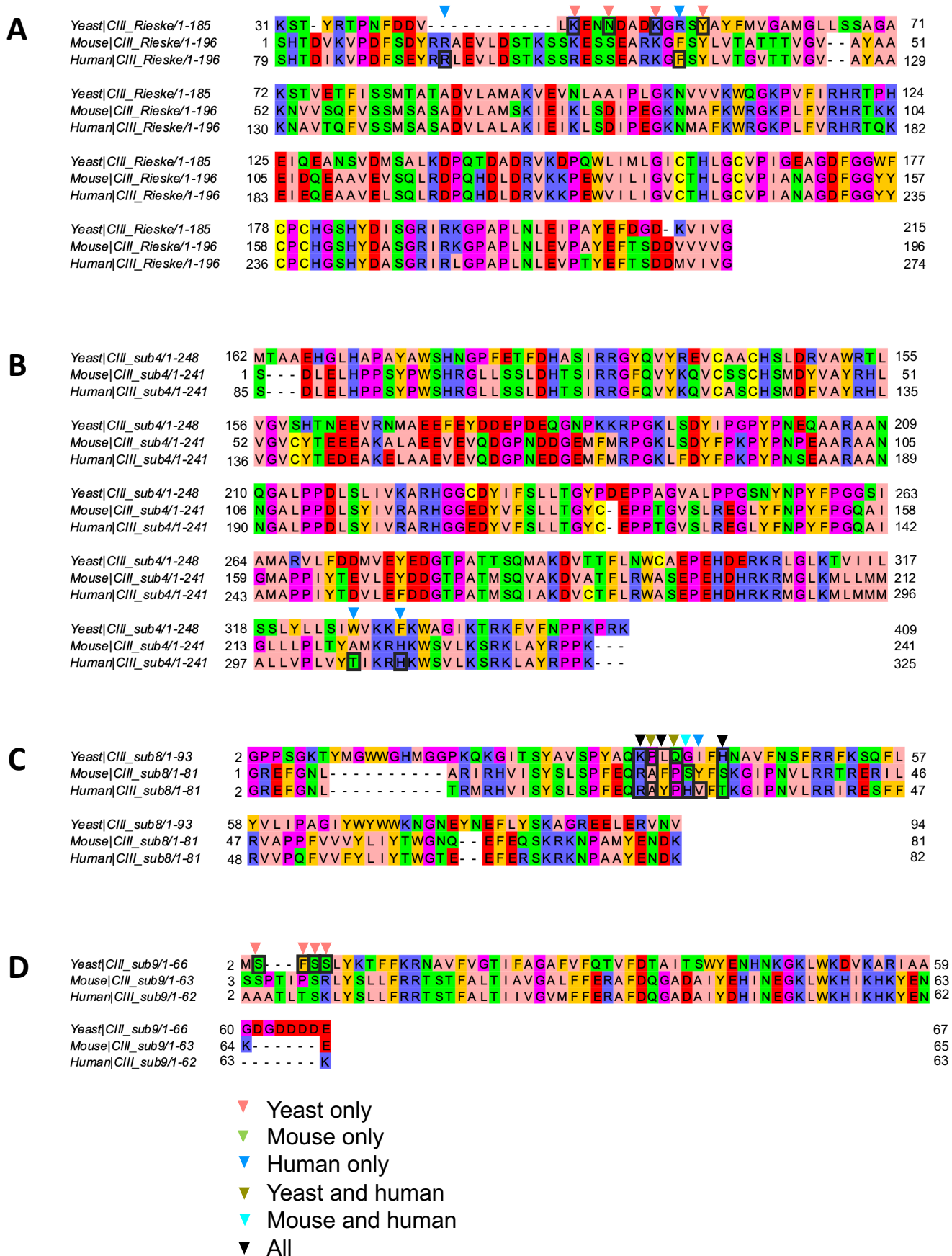


Figure S3. Sequence alignment of the yeast, mouse and human subunits of Complex III that were engaged in MLCL or CL interactions during simulations, showing **A)** the Rieske subunit; **B)** subunit 4; **C)** subunit 8; and **D)** subunit 9. Residues identified as being in the interface interaction site with occupancy > 40 % (see also Table 2) are indicated on the sequence alignment by a black outline and arrow (arrow colour shows the species involved in the interaction)

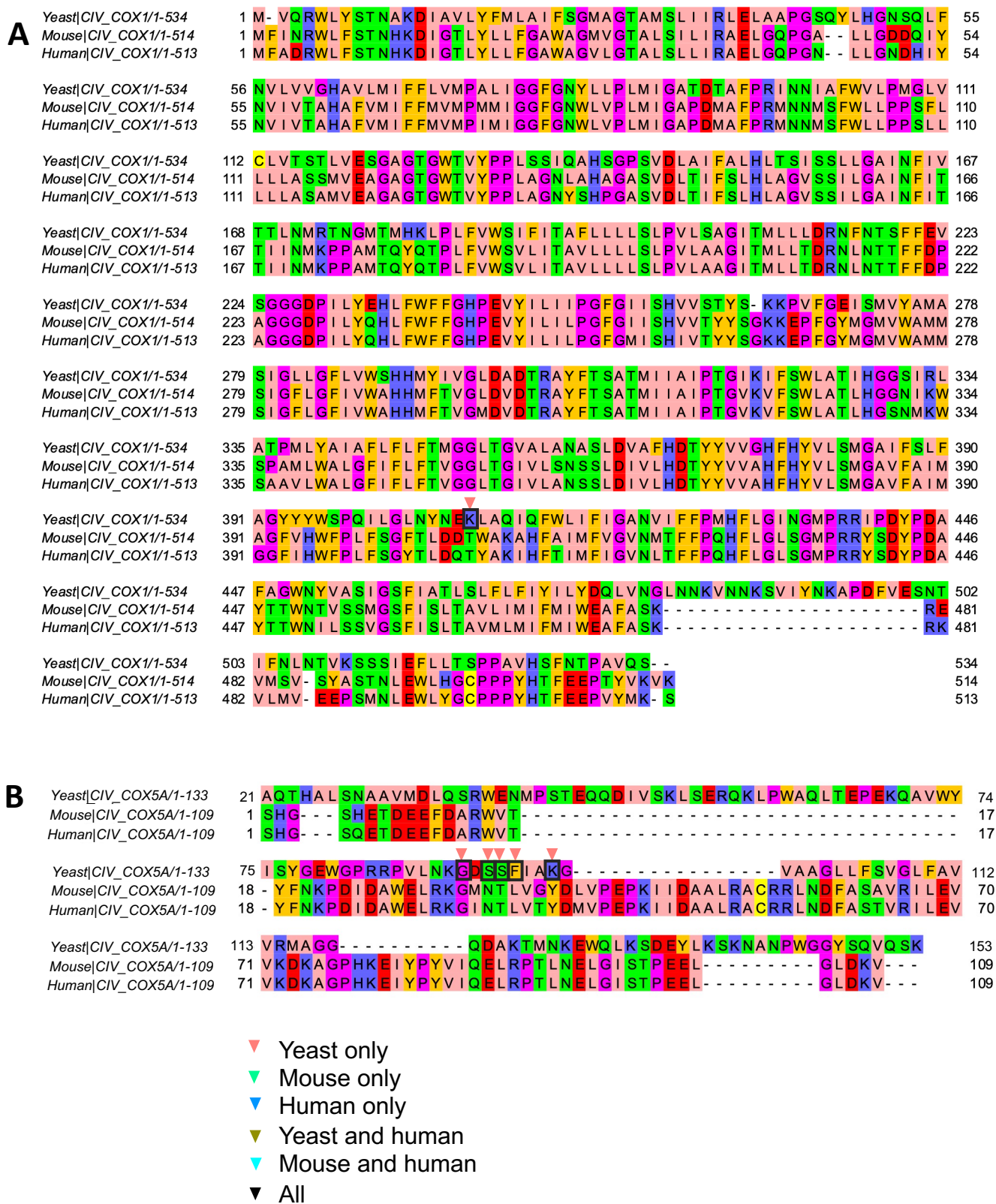


Figure S4. Sequence alignment of the yeast, mouse and human **A)** COX1; **B)** COX5A subunits of CIV. Together with Figure S5, these comprise all the subunits of CIV that were engaged in MLCL or CL interactions during simulations. Residues identified as being in the interface interaction site with occupancy > 40 % (see also Table 2) are indicated on the sequence alignment by a black outline and arrow (arrow colour shows the species involved in the interaction)

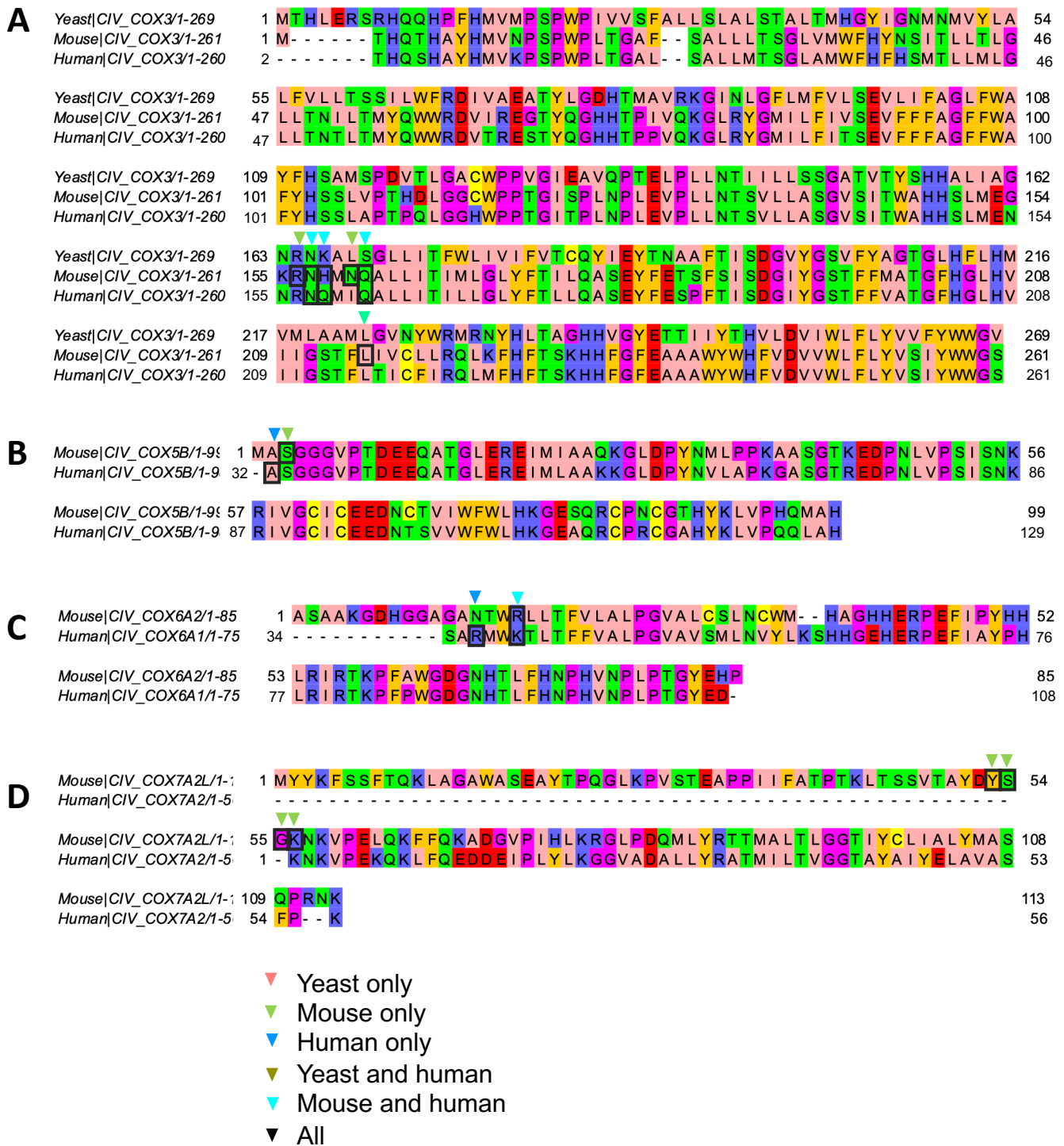


Figure S5. Sequence alignment of the yeast (*S. cerevisiae*), mouse and human **A**) COX3; and mouse and human **B**) COX5B; **C**) COX6A; and **D**) COX7A2 subunits of CIV. Note that yeast CIV does not contain subunits COX5B, COOX6A, nor COX7A2. Together with Figure S4, these comprise all the subunits of CIV that were engaged in MLCL or CL interactions during simulations. Residues identified as being in the interface interaction site with occupancy > 40 % (see also Table 2) are indicated on the sequence alignment by a black outline and arrow (arrow colour shows the species involved in the interaction)

Table S1. Residence time and occupancy for each interface site overall, and individually for each residue within the site, as listed in Table 2

Lipid	Complex and Subunit	Residue	Res. time (μ s)	Occupancy (%)	Lipid	Complex and Subunit	Residue	Res. time (μ s)	Occupancy (%)				
Yeast					Mouse								
CL (Site 1)					CL								
			Overall: 11.14	98.46				Overall: 15.00	100.00				
	CIII	Rieske	K44	8.87	48.57		CIII	Sub 8	R24	8.18	71.74		
			K51	1.91	73.51				F26	6.36	62.03		
		Sub 8	K35	3.89	42.83				S28	6.23	65.56		
		Sub 9	S2	0.69	84.99				S31	2.71	56.95		
			F3	0.59	43.05		CIV	COX3	R156	2.21	48.79		
			S4	0.63	53.20				N157	15.00	89.18		
			S5	0.70	66.89				H158	8.07	66.67		
	CIV	COX5A	S93	7.48	45.48				Q161	7.71	60.93		
			F94	3.92	43.49				L223	14.43	59.16		
			K97	3.89	40.40			COX5B	S3	8.19	71.08		
								COX6A2	R17	6.55	56.51		
								COX7A2L	S54	2.59	75.94		
									G55	2.71	62.69		
									K56	1.97	96.47		
CL (Site 2)					MLCL								
			Overall: 12.05	100.00				Overall: 8.48	99.12				
	CIII	Rieske	K44	3.78	47.68		CIII	Sub 8	R24	7.11	41.72		
			K51	1.25	74.17				S31	0.56	50.33		
			W55	3.91	49.23				R156	15.00	76.60		
		Sub 8	K35	9.80	81.02		CIV	COX3	N157	9.02	83.22		
			P36	8.55	55.85				H158	7.17	42.61		
			L37	9.06	47.02				N160	15.00	58.50		
		Sub 9	S2	0.95	93.38				L223	15.00	81.02		
			S4	0.81	52.32				COX5B	S3	7.10	41.50	
			S5	2.17	66.89				COX7A2L	Y53	2.29	9.27	
	CIV	COX5A	S93	7.71	49.45				S54	15.00	90.51		
			K97	9.27	71.97				G55	15.00	80.57		
									K56	10.53	96.69		
MLCL (Site 1)					Human								
			Overall: 10.21	93.84	CL								
	CIII	Rieske	K44	4.36	76.91				Overall: 15.00	100.00			
			N46	2.03	41.96		CIII	Rieske	R93	15.00	87.20		
			K51	1.66	68.77				F113	15.00	77.70		
		Sub 8	K35	6.12	63.95			Sub 4	T305	15.00	41.72		
	CIV	COX1	K408	2.36	46.13				H309	15.00	93.38		
		COX5A	G90	1.98	41.71			Sub 8	R25	15.00	97.79		
			F94	4.08	59.98				A26	15.00	65.12		
			K97	6.49	61.52				Y27	15.00	63.36		
MLCL (Site 2)					MLCL								
			Overall: 15.10	100.00				Overall: 15.00	100.00				
	CIII	Rieske	K44	14.06	73.82				P28	2.29	49.67		
			N46	14.36	49.40				H29	3.13	56.73		
			K51	1.24	71.42				V30	1.71	43.27		
			Y55	6.64	61.75				T32	2.37	59.16		
		Sub 8	K35	13.30	94.28		CIV	COX3	N157	3.02	75.94		
			P36	3.94	48.11				Q158	15.00	83.44		
			L37	3.68	46.74				A32	15.00	83.22		
			Q38	8.94	45.42			COX5B	R36	15.00	81.24		
			H42	1.60	47.71			COX6A1	K39	15.00	95.81		
	CIV	COX5A	G90	6.90	50.33				Overall: 15.00	100.00			
			S92	3.69	48.17				CIII	Rieske	R93	15.00	90.95
			S93	7.06	71.20						F113	15.00	79.03
			F94	7.07	72.09					Sub 4	T305	15.00	43.27
			K97	12.81	87.01						H309	15.00	92.27
										Sub 8	R25	15.00	98.23
											A26	15.00	52.76
											Y27	14.55	60.71
											P28	3.46	57.62
											H29	15.00	69.32
											V30	1.44	47.02
											T32	2.04	65.56
							CIV	COX3	N157	3.82	75.28		
									Q158	15.00	80.57		
									Q161	2.35	44.15		
									COX5B	A32	15.00	89.40	
									COX6A1	R36	15.00	87.20	
											K39	15.00	95.14