

Supplementary information

Inherent flexibility of CLIC6 revealed by crystallographic and solution studies

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Supplementary Figure legends

Supplementary Figure 1. Asymmetric unit of the C1 2 1 form and crystal packing comparison. (a)

Cartoon representation of the asymmetric unit from the C1 2 1 crystal form showing chain A and B in cyan and green, respectively. **(b and c)** Crystal packing of mCLIC6, crystallized in P2₁ and C1 2 1 space groups, respectively. A single asymmetric unit is colored as in **(a)**.

Supplementary Figure 2. Spatial comparison of mCLIC6 and selected available CLIC structures.

(a) Superposition of mCLIC6 with CLIC1 (PDB 1K0N), CLIC2 (PDB 2PER), CLIC3 (PDB 3KJY), and CLIC4 (PDB 2AHE). **(b)** All atom superposition r.m.s.d. values of the corresponding structures.

Supplementary Figure 3. Sequence similarity of mCLIC6 to human CLICs.

Sequence alignment of mCLIC6 and human members of the CLIC family. Secondary structure of mCLIC6 is indicated above the sequence. Loops and non-helical secondary structure are marked as solid gray lines. Identical and similar residues are shown in green and grey, respectively.

Supplementary Figure 4. TSA analysis of mCLIC6 in the presence of DTT.

Normalized fluorescence-temperature relation of mCLIC6 in the presence of increasing concentrations of H₂O₂ and 5 mM DTT. Boltzmann fits used to calculate *T*_m are colored according to H₂O₂ concentrations.

Supplementary Figure 5. Circular dichroism analysis of mCLIC6.

Far-UV CD spectra recorded in the absence or presence of 2 mM H₂O₂.

Supplementary Figure 6. mCLIC6 cancer-associated positions conservation.

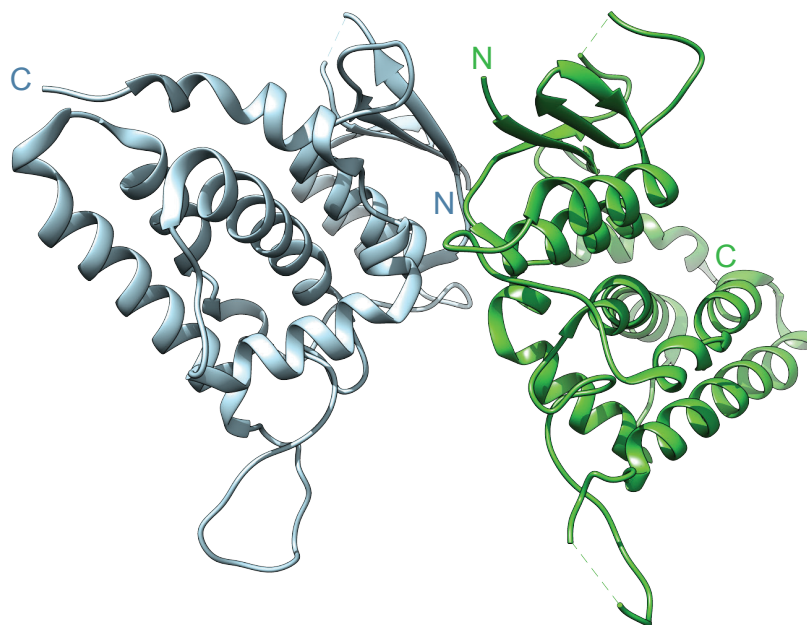
Cancer associated positions in mCLIC6 colored according to ConSurf scores.

Supplementary Figure 7. Uncropped protein gel image.

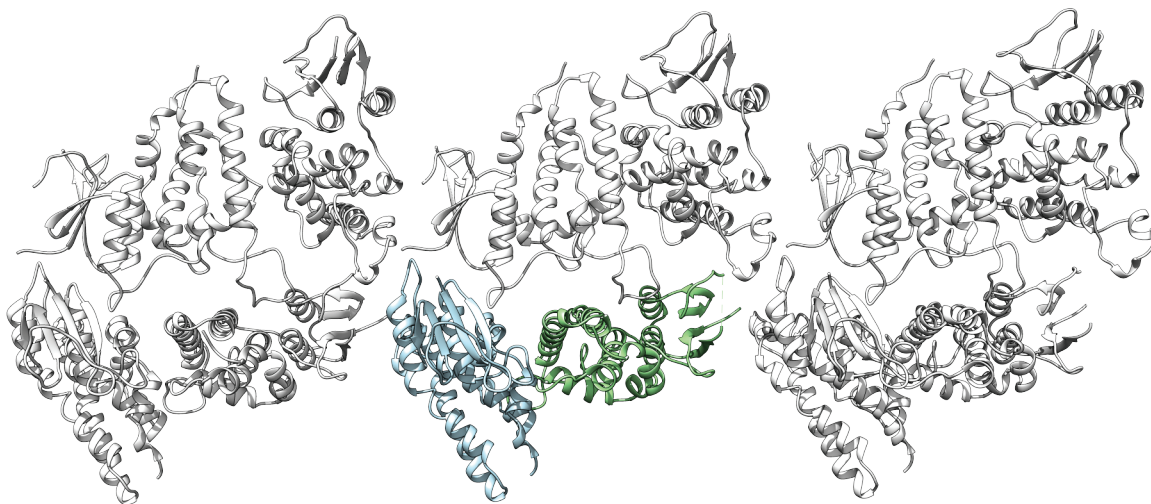
Uncropped SDS-PAGE Tris-Glycine (4-20%) protein gel image, used to produce Figure 6f.

Ferofontov et al. Supplementary Fig. S1

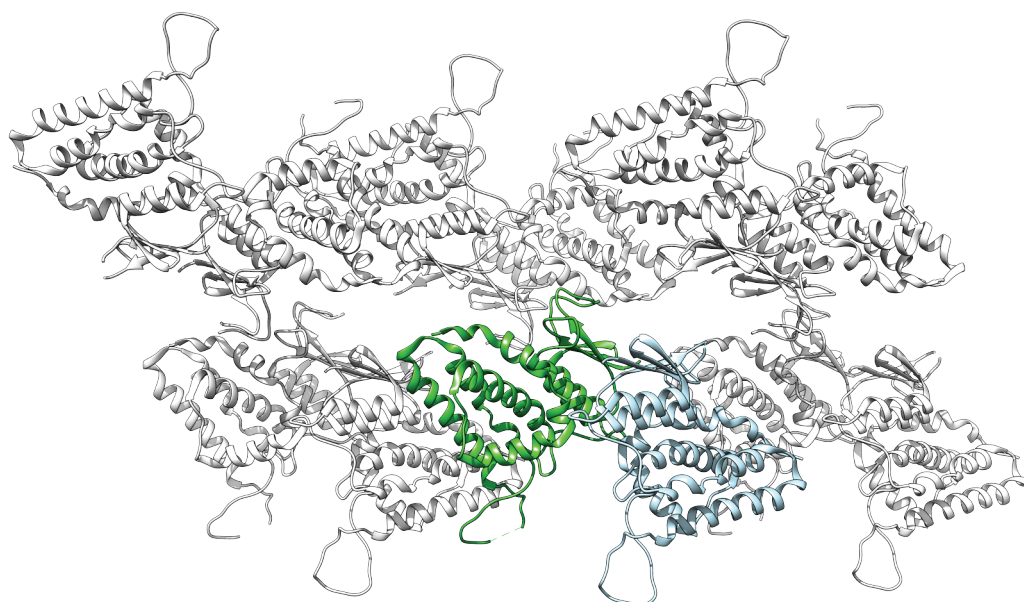
a



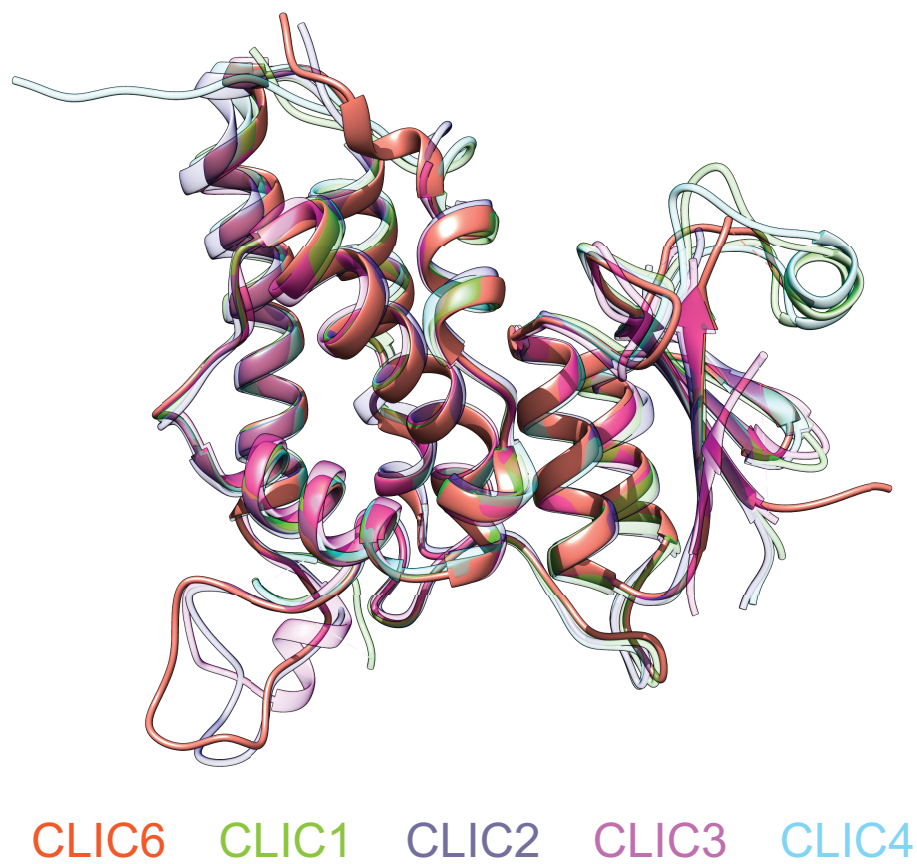
b



c



a

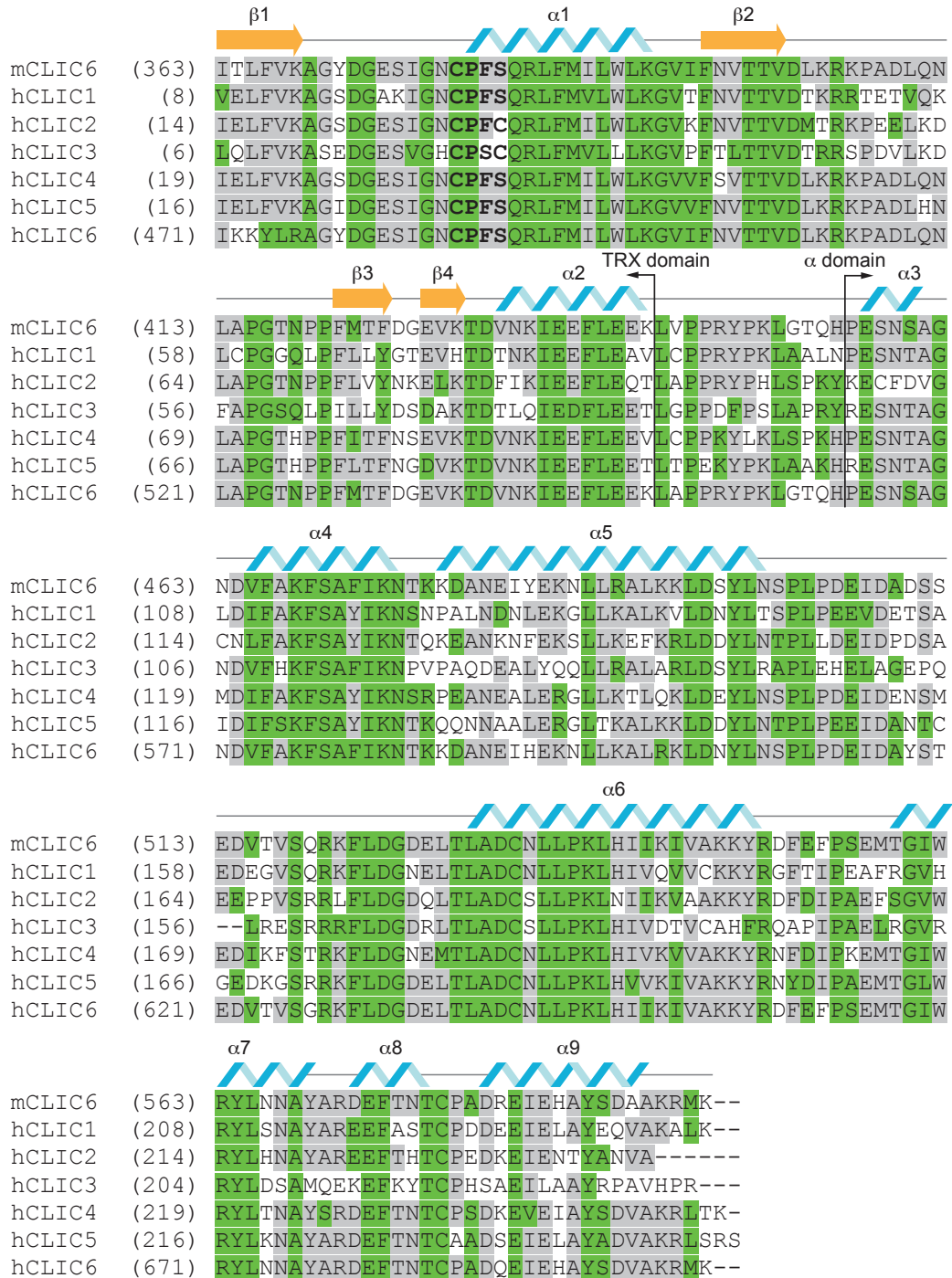


b

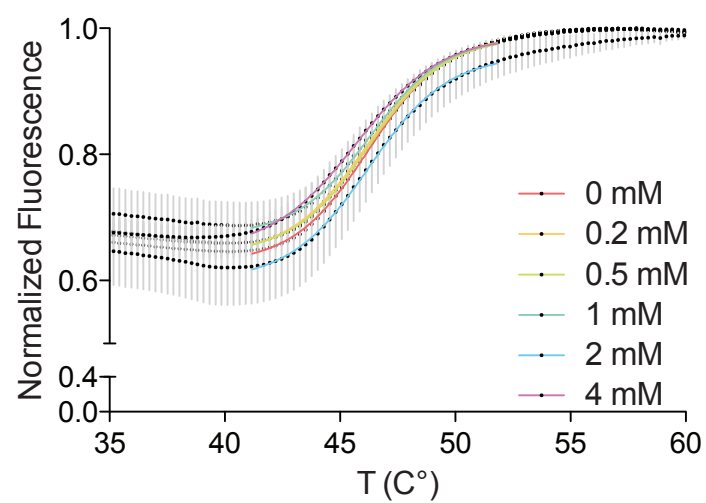
	CLIC6	CLIC1	CLIC2	CLIC3
CLIC6				
CLIC1	1.93			
CLIC2	1.83	1.15		
CLIC3	1.46	1.29	2.13	
CLIC4	1.74	0.73	0.88	1.41

Units = Å

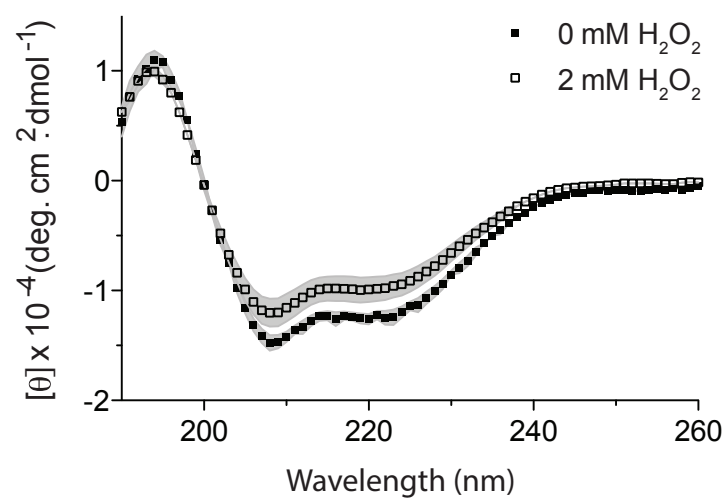
Ferofontov et al. Supplementary Fig. S3



Ferofontov et al. Supplementary Fig. S4



Ferofontov et al. Supplementary Fig. S5



Ferofontov et al. Supplementary Fig. S6

Position hCLIC6	Position mCLIC6	Human isoform	Mutation	Consurf score	Key
463	373	A	G>S	-0.218	Variable 1
467	377	A	G>E	-0.562	
472	382	A	S>Y	-0.645	
474	384	A,B	R>H	-0.949	
475	385	A,B	L>P	-0.404	
479	389	A,B	L>F	-0.538	
492	402	A,B	D>N	-0.979	
498	408	A	A>T	1.134	
499	409	A	D>V	0.065	Conserved 9
504	414	A,B	A>T	-0.652	
507	417	A,B	T>K	-0.121	
516	426	A,B	G>D	1.59	
524	434	A,B	K>T	-0.82	
526	436	A	E>K	-0.562	
539	449	A	P>A	-0.042	
545	455	A	H>R	0.125	
546	456	A,B	P>S	0.636	
547	457	A	E>K	-0.504	
550	460	A	S>C	-0.04	
551	461	A,B	A>T	-0.454	
555	465	A	V>M	-0.146	
559	469	A,B	F>L	-0.883	
561	471	A,B	A>V	-0.82	
566	476	A	T>M	-0.113	
572	482	A	E>K	2.75	
575	485	A	E>K	0.241	
576	486	A	K>N	0.351	
586	496	A,B	D>Y	-0.679	
591	501	A	S>C	0.916	
596	506	A	E>K	-0.895	
601	511	A	S>T	-0.229	
619	529	A,B	T>M	-0.942	
630	540	A,B	H>Y	-0.397	
641	551	A	D>E	0.823	
645	555	A,B	P>S	-1.137	
653	563	A	R>G	-0.431	
658	568	A,B	A>S A>V	-1.023	
661	571	A,B	R>G R>K	0.627	
662	572	A,B	D>N	0.092	
663	573	A,B	E>A	-0.837	
664	574	A,B	F>L	-1.096	
673	583	A,B	E>Q	-1.19	
685	595	A,B	M>I	-0.517	

Ferofontov et al. Supplementary Fig. S7

