Supplementary Material for

Structure and Interactions of Domain C1 of Human Cardiac Myosin Binding Protein C (MyBP-C)

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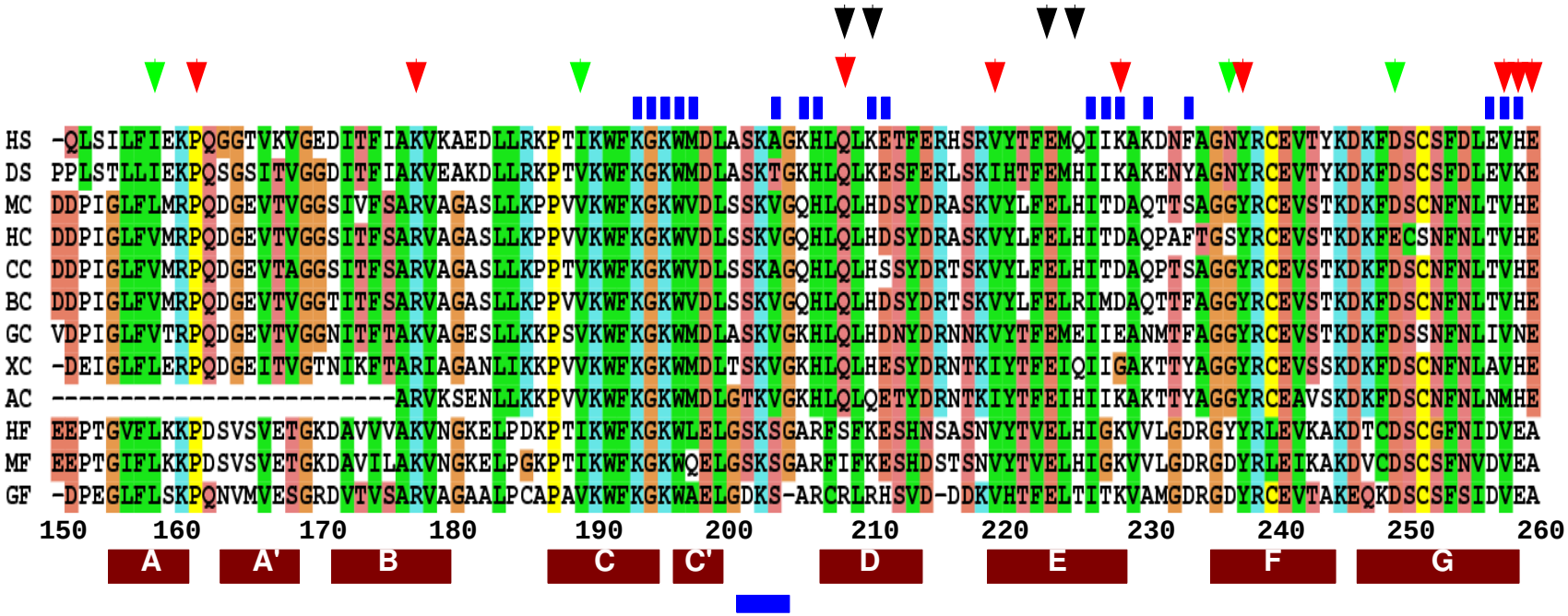

Figure S1: Alignment of C1 domains of MyBP-C. Sequence labels are: HS – Homo sapiens, slow skeletal isoform, DS – Danio rerio, slow sceletal isoform, MC - Mus musculus, cardiac isoform, HC – Homo sapiens, cardiac isoform, CC – Canis familiaris, cardiac isoform, BC – Bos Taurus, cardiac isodorm, GC – Gallus gallus, cardiac isoform, XC – Xenopus laevis, cardiac isoform, AC – Ambystoma mexicanum, cardiac isoform, HF – Homo sapiens, fast skeletal isoform, MF – mus musculus, fast skeletal isoform, GF – gallus gallus, fast skeletal isoform. Sequence conservation is indicated by colouring (Red: negative charge; Blue: positive charge, Green: hydrophobic, Pink: hydrophilic, Orange: glycine, Yellow: special residues proline or cystein). Thresholds for colouring are identical to those used previously. The amino acids in the zinc binding site are indicated by black arrows, amino acids in the binding site for S2Δ by blue squares, residues mutated in FHC by red arrows, polymorphisms by green arrows, the C1 specific insertion by a blue bar at the bottom and the β-strands in the NMR structure by brown bars. Residue numbering is for human cardiac MyBP-C.

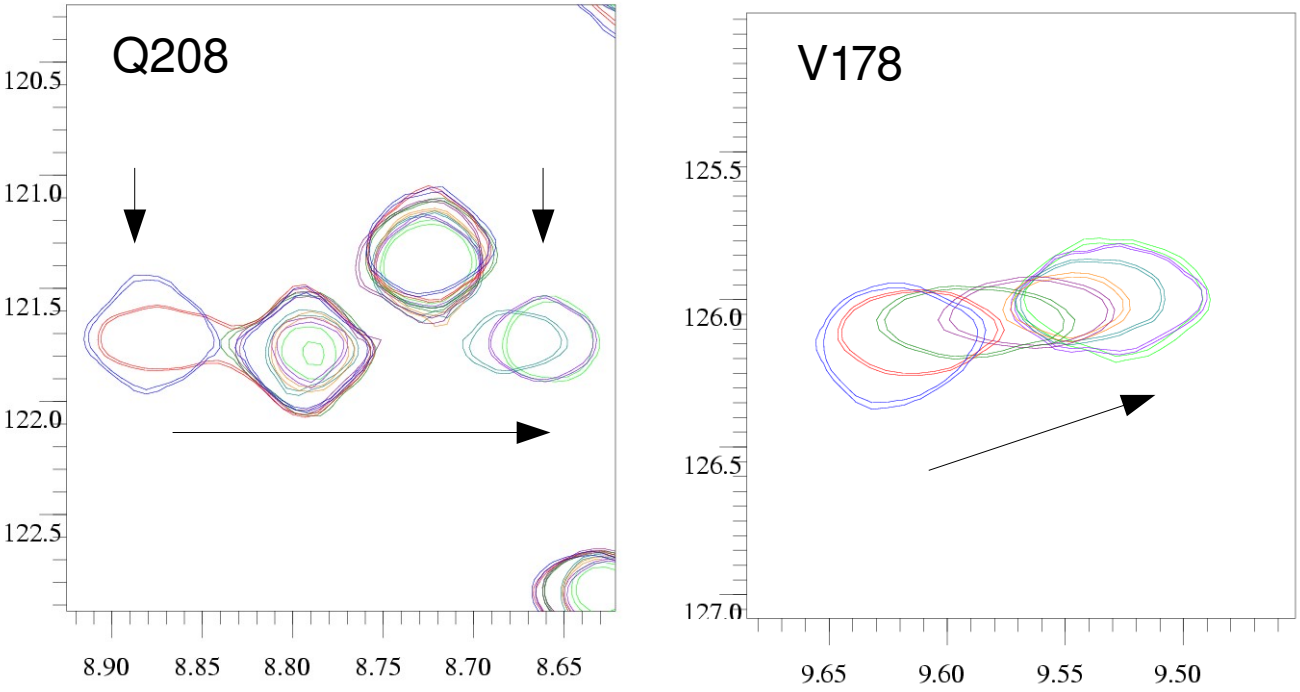

Figure S2: Zn titration of C1. Parts of the HMQC spectrum indicating the chemical shift variations of a number of residues. On the left is Q208 as an example for a residue in slow exchange and on the right V178 as an example of a residue in fast-intermediate exchange. The arrows indicate the direction of the chemical shift change in the course of the titration.

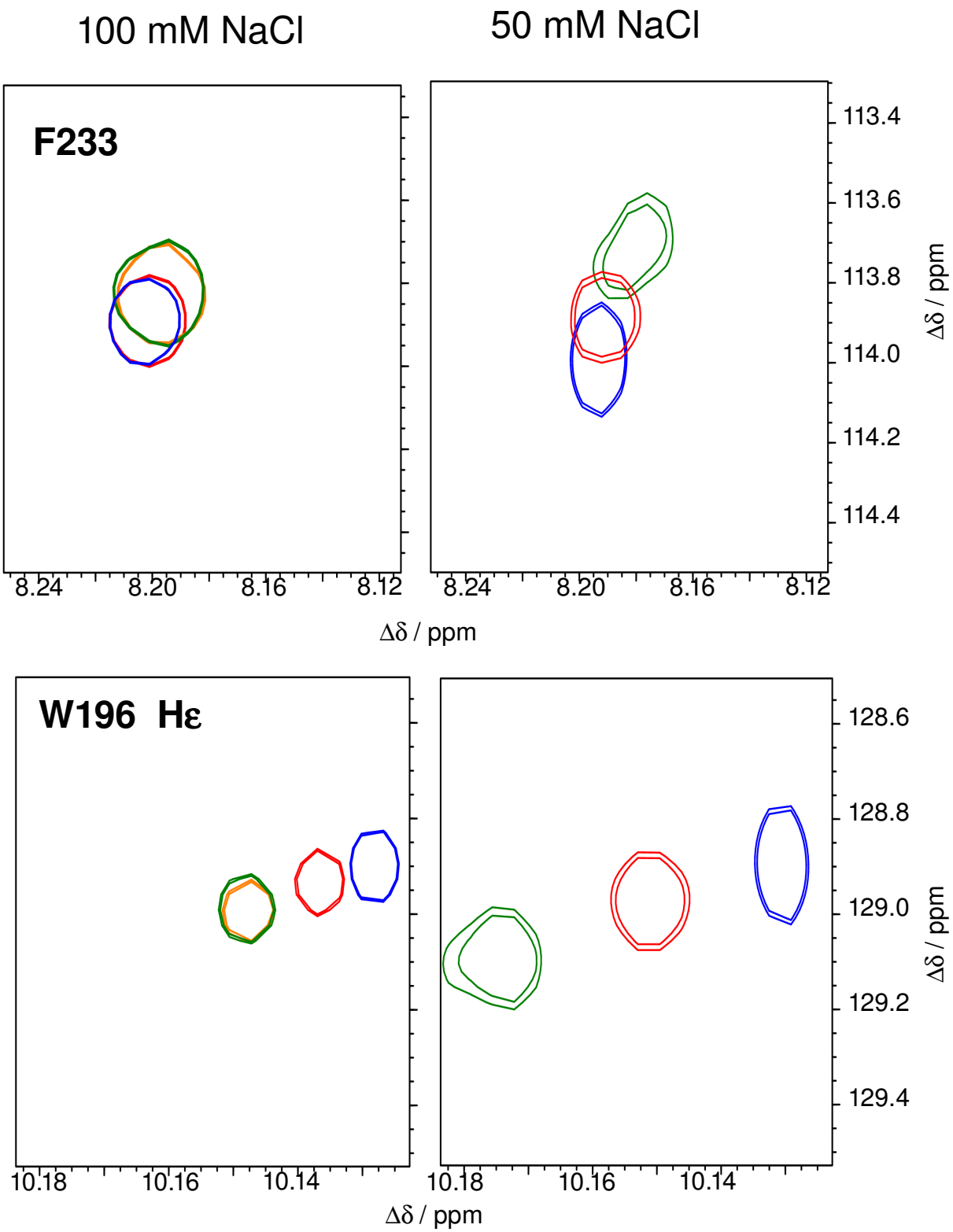

Figure S3: Chemical shift perturbations of spectra of C1 by binding to S2Δ. Spectra were recorded in 100 mM salt (left) compared to spectra in 50 mM salt (right). C1 alone (blue), C1 + S2Δ (orange), C1 + S2Δ E846K (red) and C1 + S2Δ E894G (green).

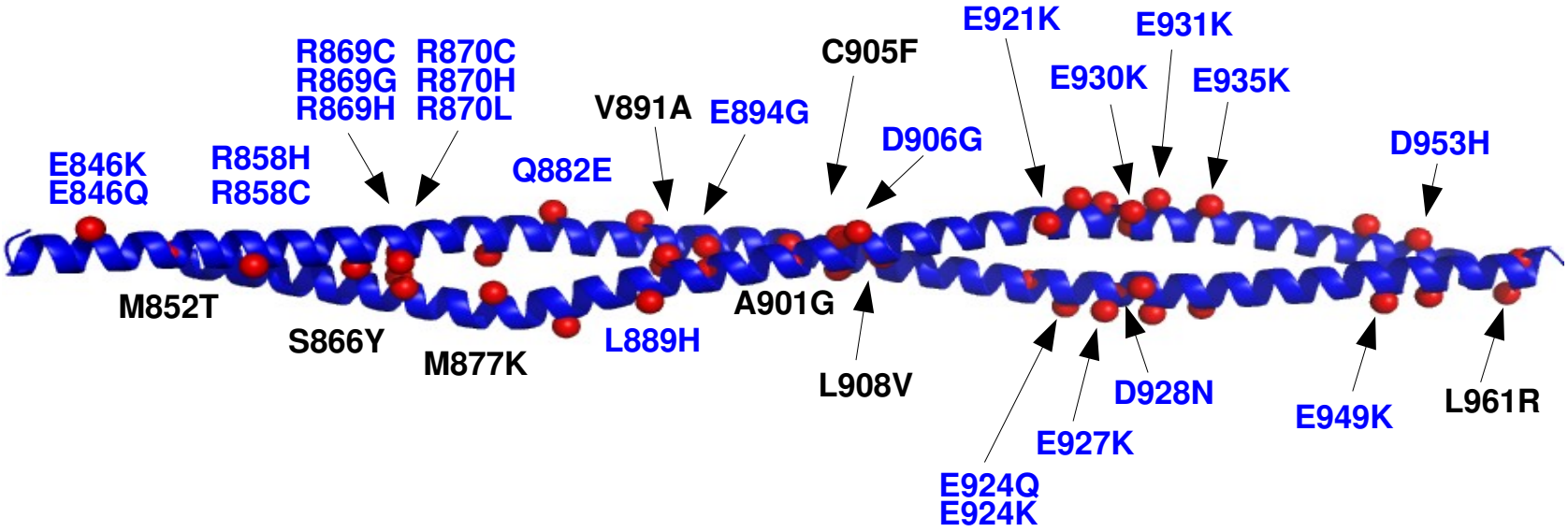

Figure S4: Mapping the location of FHC related point mutations on the structure of the myosin fragment S2Δ. Labels for solvent exposed residues are coloured in blue, for non-exposed residues in black. Note that there is so far only one polymorphism for this part of myosin (R858G) so that no explicit labelling was performed here.

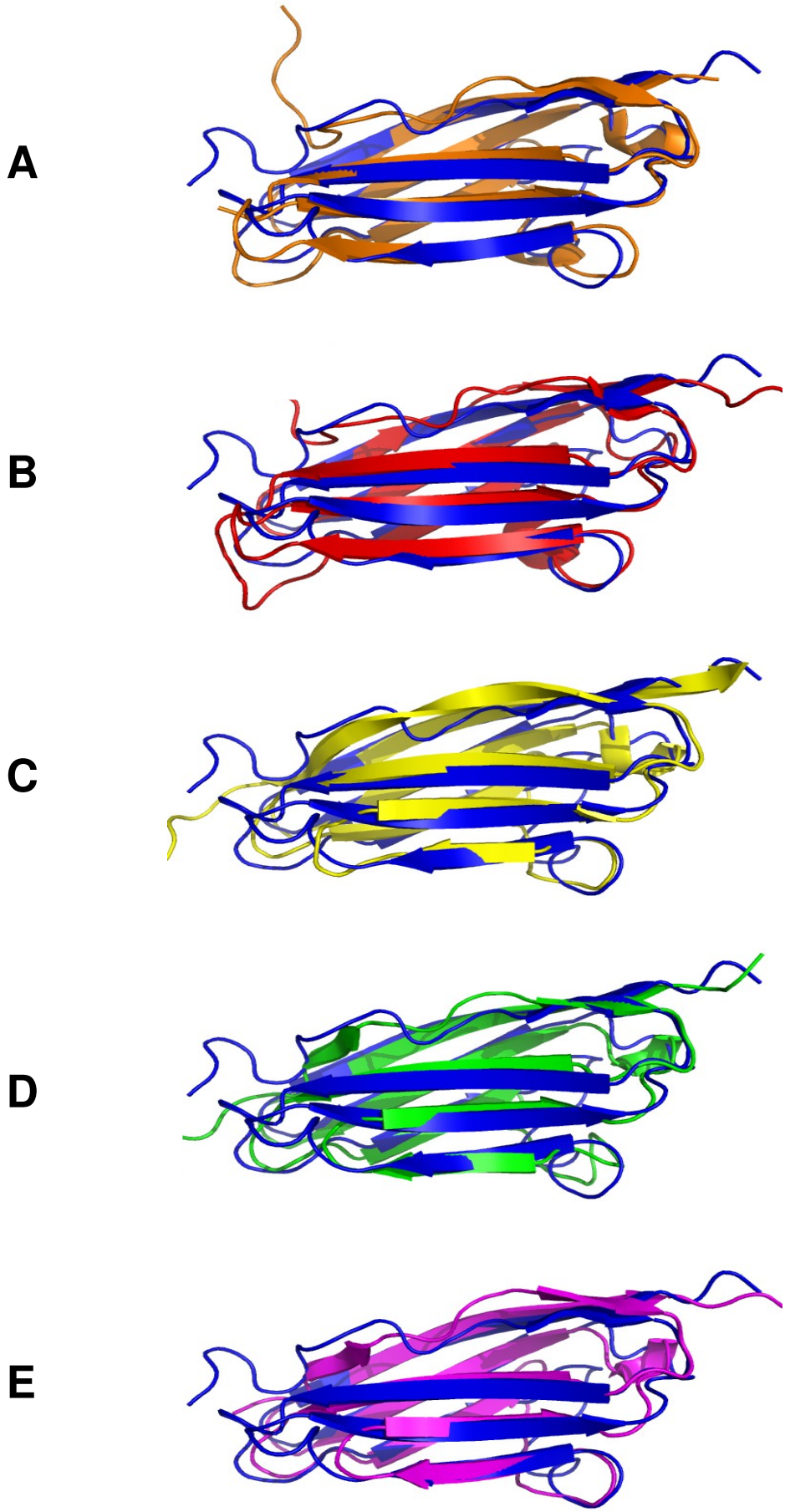

Figure S5: Comparison of the NMR structure of human cardiac C1 with related structures. The NMR structure of the cardiac isoform of C1 is shown in blue throughout. It is superimposed on the X-ray structure (A, 3CX2, orange), the NMR structure of human slow skeletal C1 (B, 2DAV, red),the X-ray structure of telokin (C, 1TLK, colour), the X-ray structure of human cardiac titin domain Z1 (D, 1YA5, green) and Z2 (E, 1YA5, magenta). Structures were superimposed with DALI LITE.

PDB ID	Z-score	RMSD / Å	Seq Ident / %	protein
3CX2	13.2	1.8	99	X-ray structure of cardiac C1
2DAV	11.2	2.4	53	Slow skeletal human MyBP-C, C1
1TLK	10.3	2.5	22	Telokin
1YA5-N	10.1	2.2	22	Titin, domain Z1
1YA5-C	10.5	2.0	24	Titin, domain Z2

Table S1: Sequence and structure similarities of C1 to other IgI type domains as calculated with DALI-Lite. Sequence identity is calculated for structurally aligned sequences.