Supporting information:

The intrinsically disordered carboxy-terminus of troponin-T binds to troponin-C to modulate myocardial force generation

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Contents:

Table S1. In silico prediction tools applied to *TNNC1*-p.Ile4Met

	MutPred2	PolyPhen-2	MutationTaster	SIFT
Prediction	Pathogenic	Benign	Disease-causing	Tolerated

Predictions were performed on MutPred2 (1), PolyPhen-2 (2), MutationTaster (3), and SIFT (4) using the FASTA sequence (with Ile4Met substitution) for human *TNNC1* (UniProt P63316) or Ensemble transcript ID (ENST00000232975). MutPred2 score: 0.569. MutationTaster probability: 0.999.

Table S2. cTnT-cTnI intersubunit cross-linked residues

cTnT	cTnI
K197	K117
K197	K120
K200	K117
K207	K117
K207	K120
K255	K117
K258	K117
K258	K120
K273	K138

Verified cTnT and cTnI intersubunit BS3 cross-links identified in the “dark band” (Fig. 5A).

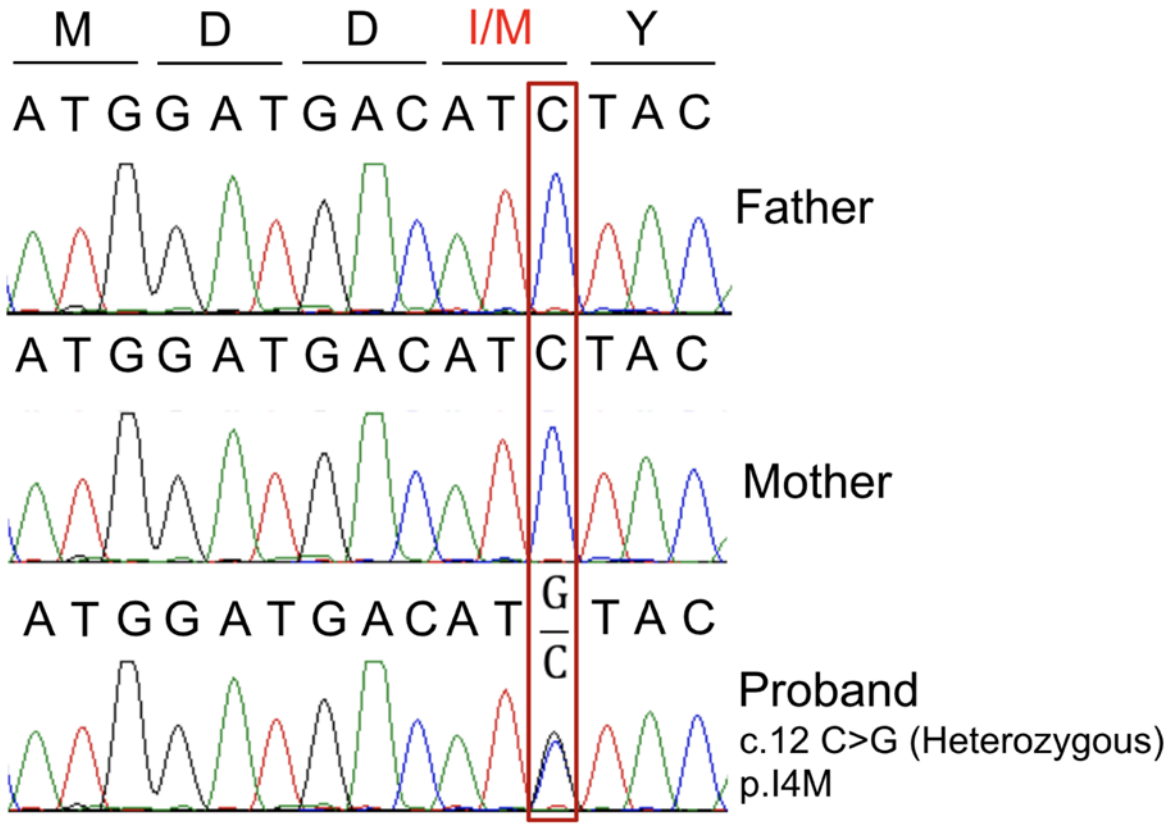

Fig. S1. Trio whole exome sequencing chromatograms. The first 5 codons in exon 1 of *TNNC1* are shown with their respective amino acid residues (single letter notation). Red box indicates the position at which the substitution occurred in the proband. Tracings: green, adenine (A); red, thymine (T); black, guanine (G); blue, cytosine (C). Note the presence of signals from both G and C at the 12th nucleotide position (12 C>G) in the proband, indicating heterozygosity.

Fig. S2. Multiple protein sequence alignment of *TNNC1* across various species. Red box indicates the conserved isoleucine (I) at position 4 in the primary amino acid sequence.

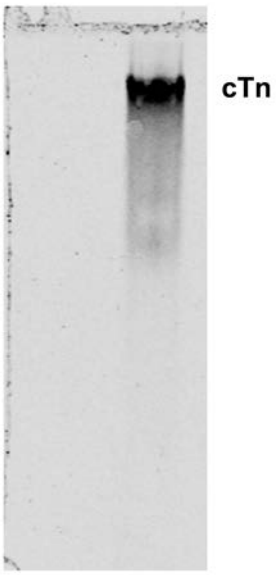

Fig. S3. Coomassie-stained native gel (4% stacking, 8% resolving) analysis of wild-type cTn.

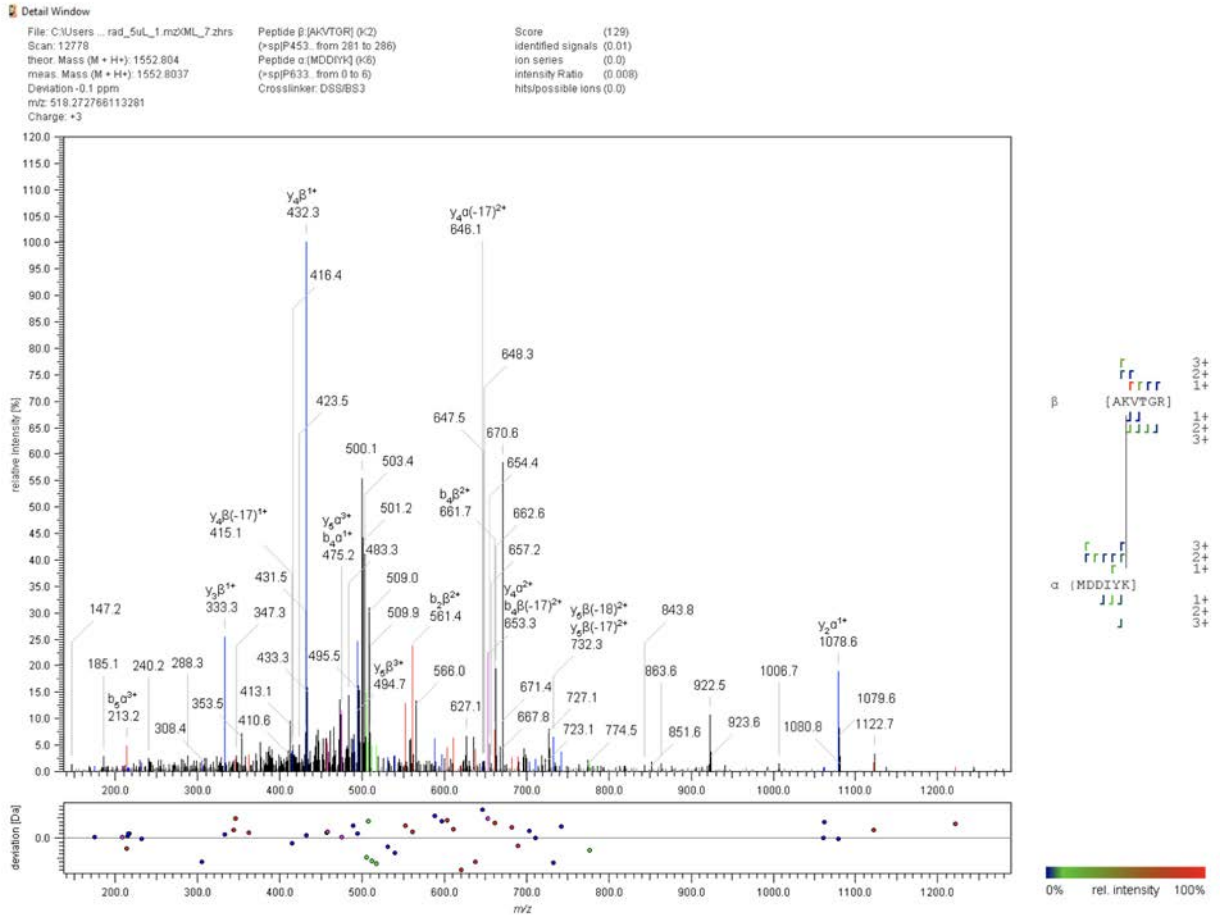

Fig. S4. Observed MS2 of the precursor ion *m/z* of 518.2721 corresponding to the cross-linked peptides between cTnT-K282 and cTnC-K6. Complete sequence coverage allows for localization of the cross-linking sites.

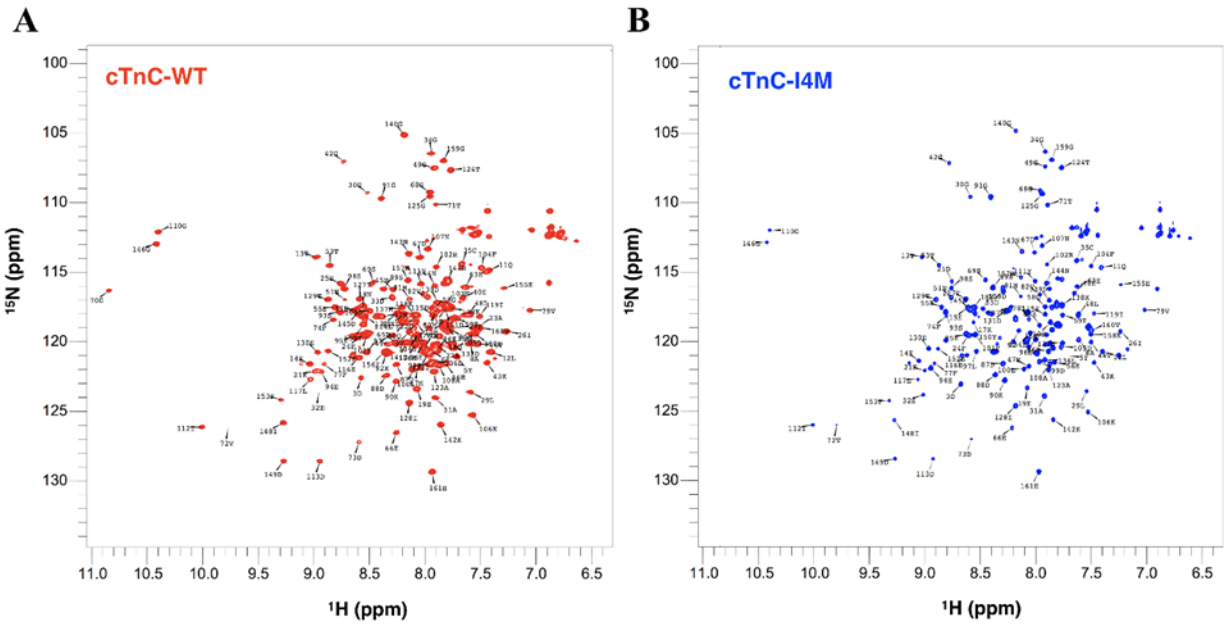

Fig. S5. Individual ^{1}H-^{15}N HSQC spectra for **(A)** cTnC-WT and **(B)** cTnC–I4M. The ^{1}H-^{15}N assignment for cTnC-I4M was obtained by cross-peak transferring from our previous cTnC-WT assignment. For further details please see Experimental Procedures.

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