Supporting Information

LIM domain-wide comprehensive virtual mutagenesis provides structural rationale for cardiomyopathy mutations in CSRP3

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Supplementary Material and Methods

Solvent accessible surface area

FreeSASA, an open-source tool was utilised for inferring total and polar solvent accessible area change due to each substitution in mutational landscape [1]. For this, high-precision calculation parameter (1000 slides per atom) was set using Lee and Richards (L&R) algorithm [2]. LIM1 and LIM2 mutations' total SASA were plotted against mutational landscape.

Contact Map Analysis

Contact map analysis of the MD trajectories can be helpful to acknowledge the lifetime of contacts across the structure or new conformational states traversed by a system. CONAN MAP [3] was utilised for this task. Only protein, excluding the TIP3P atoms, was considered from the trajectories in this analysis, and inter-residue contacts as well as correlation were calculated. All the frames of the MD trajectories were used for the study with an interval of 1 ns.

Inter Residue Contact Analysis

CONAN uses three cut-offs for inter-residue distance calculation:

r_{cut}: This is the primary cut-off value. Any residue pair without any atoms within this cut-off is disregarded.

r_{inter}: This is the cut-off value under which interactions are formed.

r_{inter}^{high} This is the cut-off value over which interactions are broken.

The inter-residue distance is defined as:

A main cut-off r_{cut}=1 nm, and interactions defined using the same cut-off, r_{inter}= r_{inter}^{high} =0.5 nm.

SI Figures

Figure S1 heatmap of solvent accessible surface area (SASA) for LIM1 and LIM2 domains of CSRP3. Reported change in local SASA due to amino acid substitution at each position (ΔÅ²) is colored in shades of green (decreased in mutant compared to WT) and pink (increased in mutant compared to WT). A) LIM1 domain SASA heatmap and B) LIM2 domain SASA heatmap.

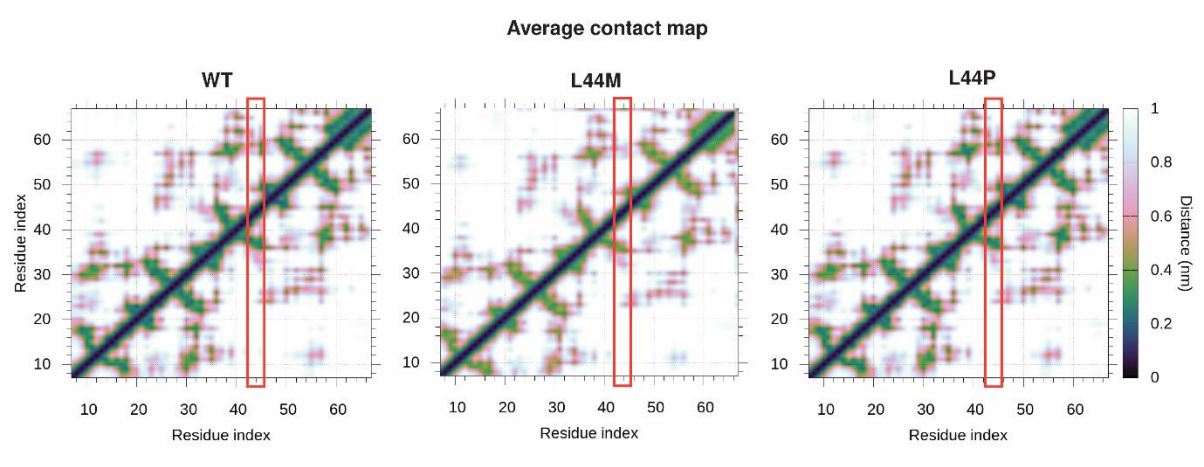
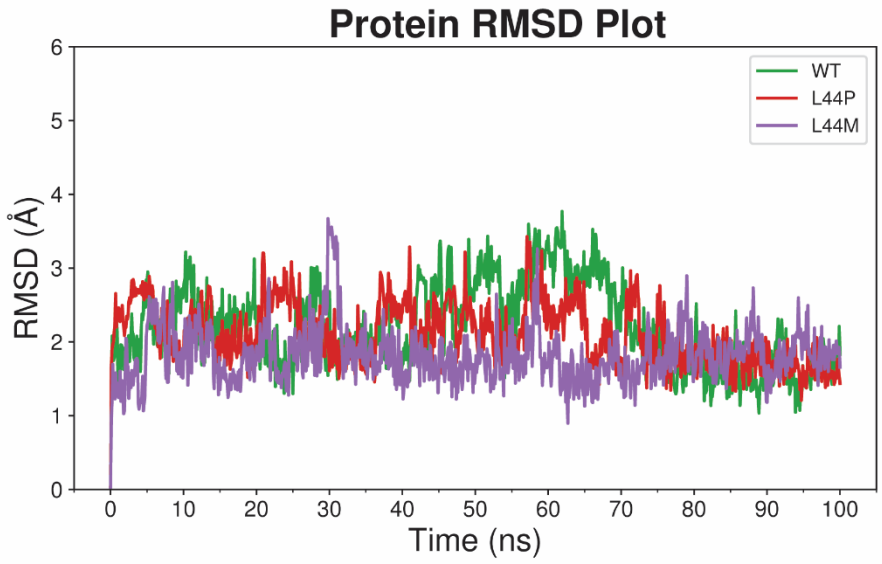
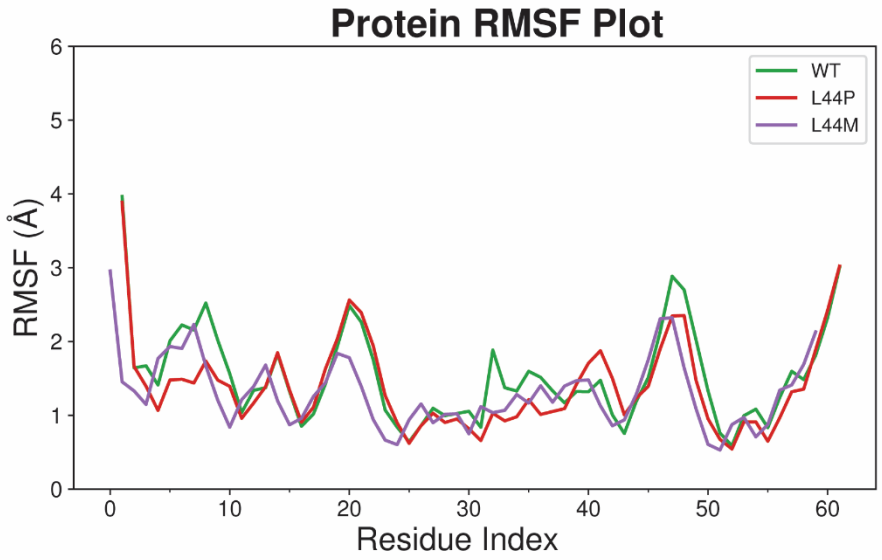


Figure S2 Contact map between residues pairs of LIM1 domain. CONAN tool default criterion was used for WT, L44M and L44P trajectories. Distance between residue pairs vary from 0-1 nm (dark blue to white color as seen in the legend). Red outlined box indicates L44 region.

A


Protein RMSD Plot
WT
L44P
L44M
RMSD (Å)
Time (ns)

B


Protein RMSF Plot
WT
L44P
L44M
RMSF (Å)
Residue Index

C

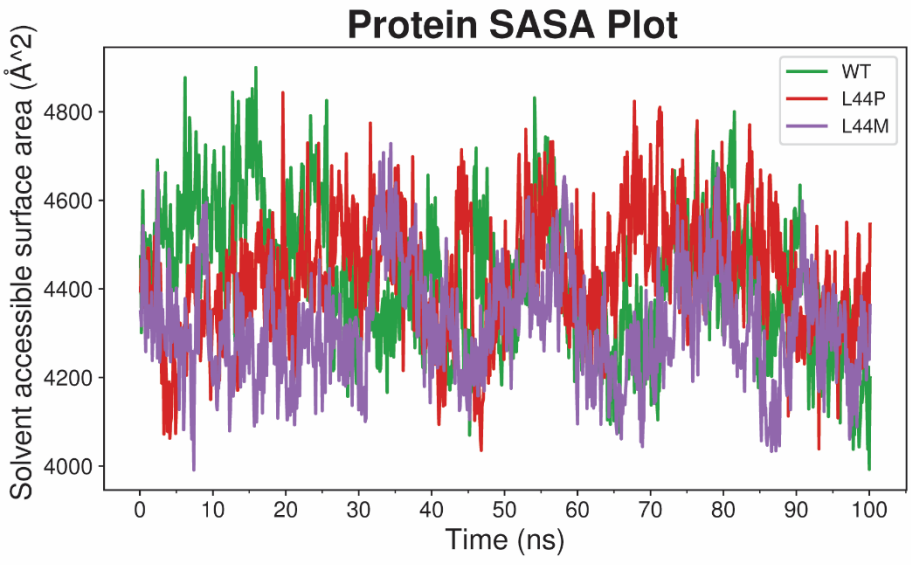

Protein SASA Plot
WT
L44P
L44M
Solvent accessible surface area (Å^2)
Time (ns)

Figure S3 Simulation event analysis of LIM1 domain. Desmond package was used for simulation event analysis of WT,L44M and L44P trajectories labelled as green (WT), indigo (L44M) and red (L44P). A) Root mean square distance (RMSD) plot of the mentioned trajectories. B) Root mean square fluctuations (RMSF) of WT, L44M and L44P. C) Solvent accessible surface area (SASA) plot of the aforesaid trajectories.

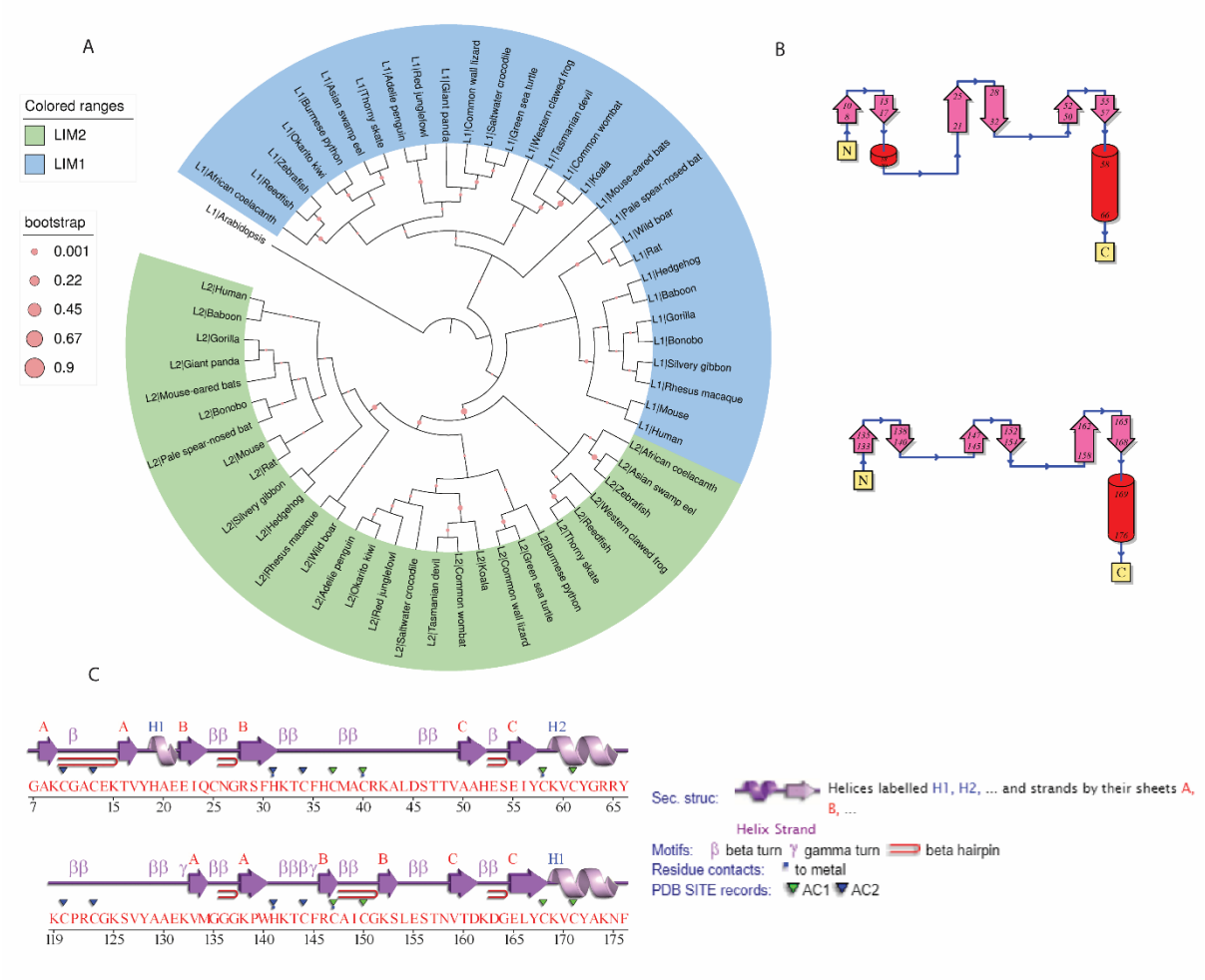


Figure S4 Sequence and structural differences between LIM1 and LIM2 domain of CSRP3. A) Maximum Likelihood Phylogeny of LIM1 and LIM2 in the representative eukaryotes with 1000 bootstrap iterations (pink circles) LIM and LIM2 are colored in blue and green respectively. B) Secondary structure connectivity map of LIM1 and LIM2 domains as seen in the pictorial database PDBsum . C) Two dimensional view of LIM domains of CSRP3 derived from PDBsum.

SI Tables

Organism	**Common name**	**Taxa**
Xenopus tropicalis	Western clawed frog	Amphibian

Gallus gallus	Red junglefowl	Aves
Pygoscelis adeliae	Adelie penguin	Aves
Apteryx rowi	Okarito kiwi	Aves
Sus scrofa	Wild boar	Carnivore
Ailuropoda melanoleuca	Giant panda	Carnivore
Danio rerio	Zebrafish	Fish
Monopterus albus	Asian swamp eel	Fish
Erpetoichthys calabaricus	Reedfish	Fish
Amblyraja radiata	Thorny skate	Fish
Latimeria chalumnae	African coelacanth	Fish
Sarcophilus harrisii	Tasmanian devil	Marsupial
Phascolarctos cinereus	Koala	Marsupial
Vombatus ursinus	Common wombat	Marsupial
Podarcis muralis	Common wall lizard	Other vertebrate
Python bivittatus	Burmese python	Other vertebrate
Chelonia mydas	Green sea turtle	Other vertebrate
Crocodylus porosus	Saltwater crocodile	Other vertebrate
Myotis myotis	Mouse-eared bats	Placental
Phyllostomus discolor	Pale spear-nosed bat	Placental
Echinops telfairi	Hedgehog	Placental
*Arabidopsis thaliana**	arabidopsis	Plant
Homo sapiens	Human	Primate
Pan paniscus	Bonobo	Primate
Macaca mulatta	Rhesus macaque	Primate
Hylobates moloch	Silvery gibbon	Primate
Gorilla gorilla	Gorilla	Primate
Papio anubis	Baboon	Primate
Mus musculus	Mouse	Rodent
Rattus rattus	Rat	Rodent

*Table S1 Representative eukaryotes used for CSRP3 sequence conservation and phylogeny analysis. *Arabidopsis was used as out-group in the study.*

References

1. Mitternacht, S. FreeSASA: An open source C library for solvent accessible surface area calculations. *F1000Research* **5**, 189 (2016).

2. Lee, B. & Richards, F. M. The interpretation of protein structures: Estimation of static accessibility. *Journal of Molecular Biology* **55**, 379-IN4 (1971).

3. Mercadante, D., Gräter, F. & Daday, C. CONAN: A Tool to Decode Dynamical Information from Molecular Interaction Maps. *Biophysical Journal* **114**, 1267–1273 (2018).