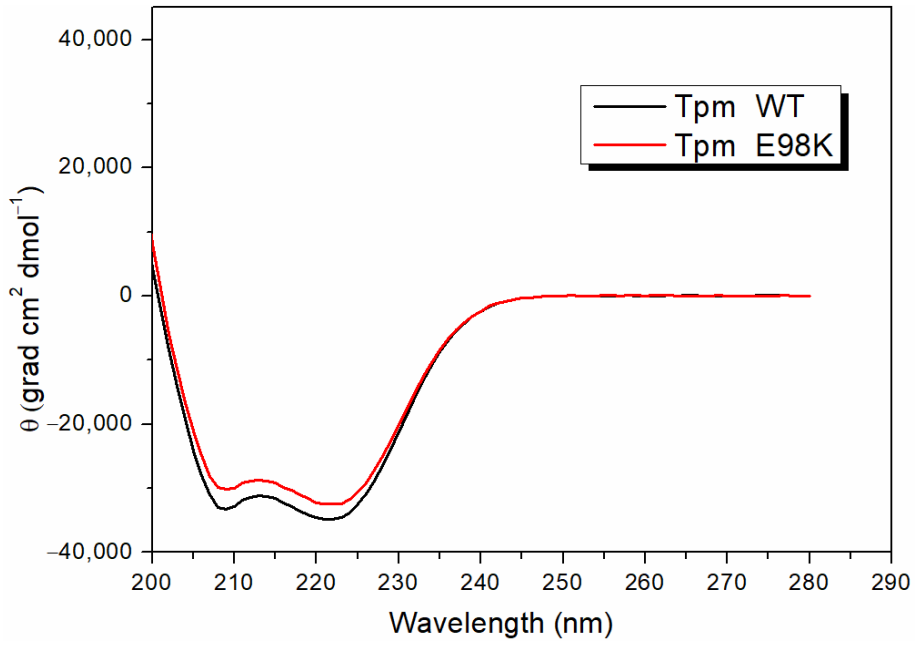

Figure S1. CD spectra of the Tpm E98K compared with Tpm WT. Spectra were recorded at 10 ^{o}C.

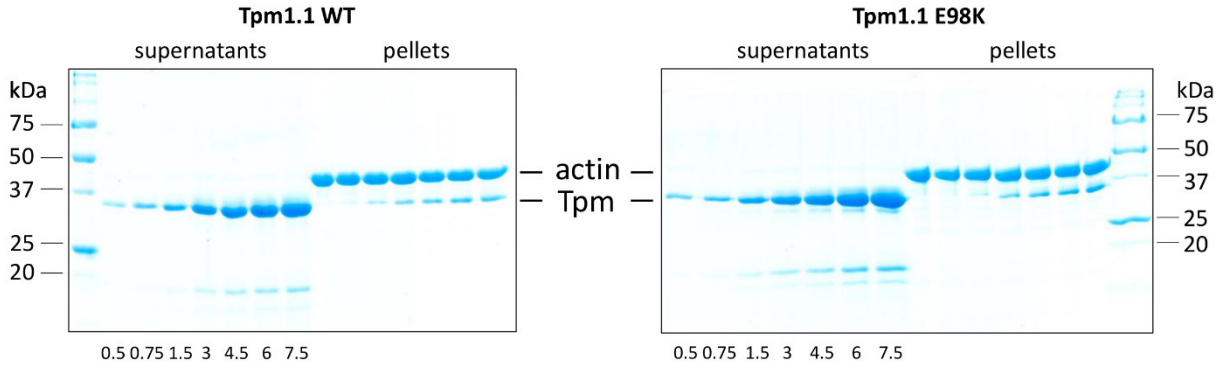

Figure S2. Representative SDS-PAAG gels showing supernatants and pellets obtained from co-sedimentation of Tpm WT and E98K Tpm with F-actin. Total concentrations (μM) of Tpm in the samples are listed under the gels. It is clear that the E98K substitution does not significantly affect the Tpm affinity to F-actin.

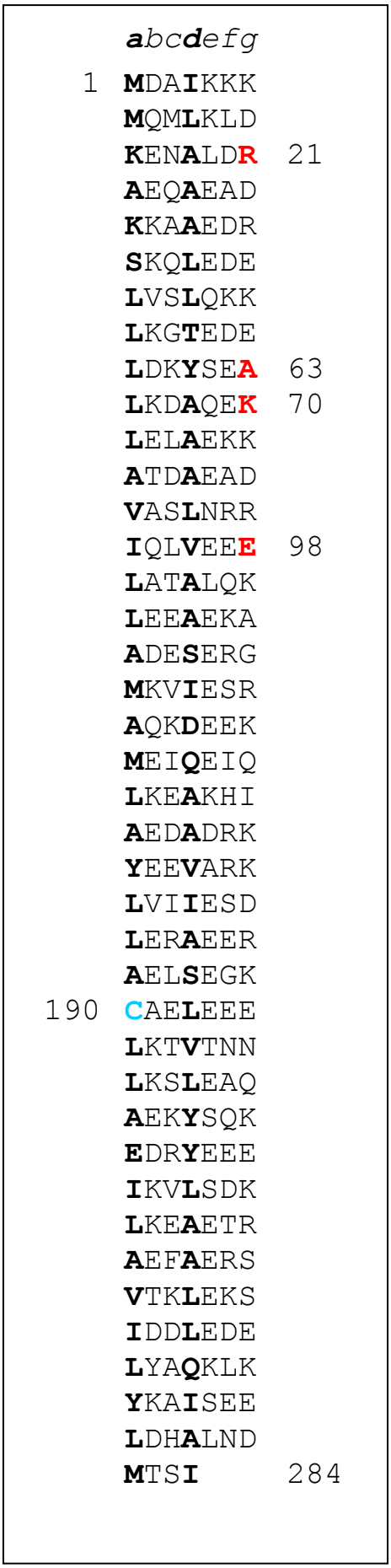

Figure S3. Heptad repeats $(abcdefg)_n$ in the amino acid sequence of cardiac Tpm1.1 isoform. Hydrophobic residues at positions *a* and *d* that form a hydrophobic core of the molecule are shown in bold.
The residues R21, A63, K70, and E98 at *g* position in the N-terminal part of Tpm, whose substitutions with His, Val, Thr, and Lys residues, respectively, by HCM-associated mutations R21H, A63V, K70T, and E98K in the *TPM1* gene are shown in red. The only Cys residue of Tpm1.1 (C190) is shown in blue.