Protein thermodynamic destabilization in the assessment of pathogenicity of a variant of uncertain significance in cardiac myosin binding protein C

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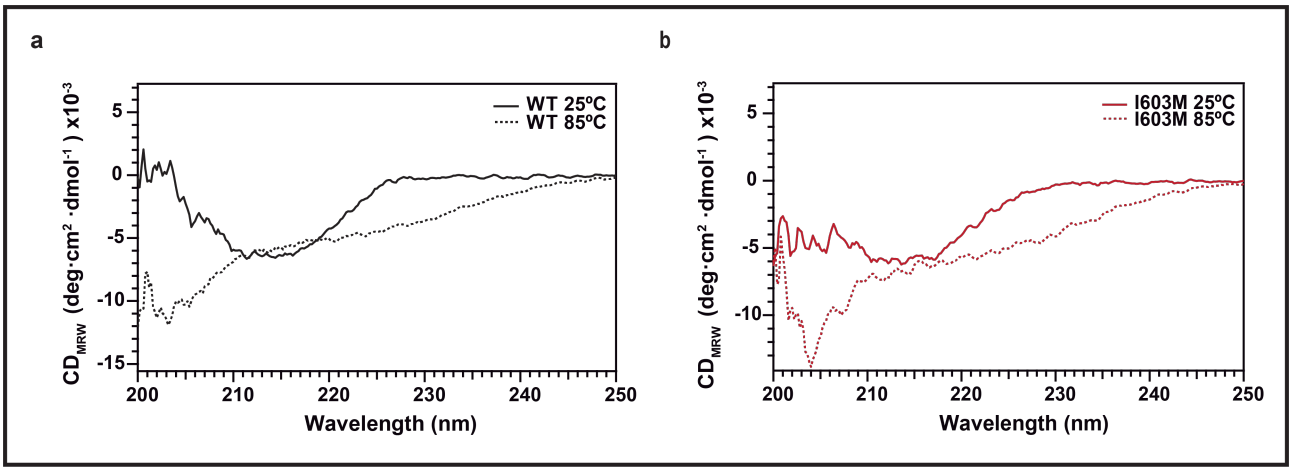
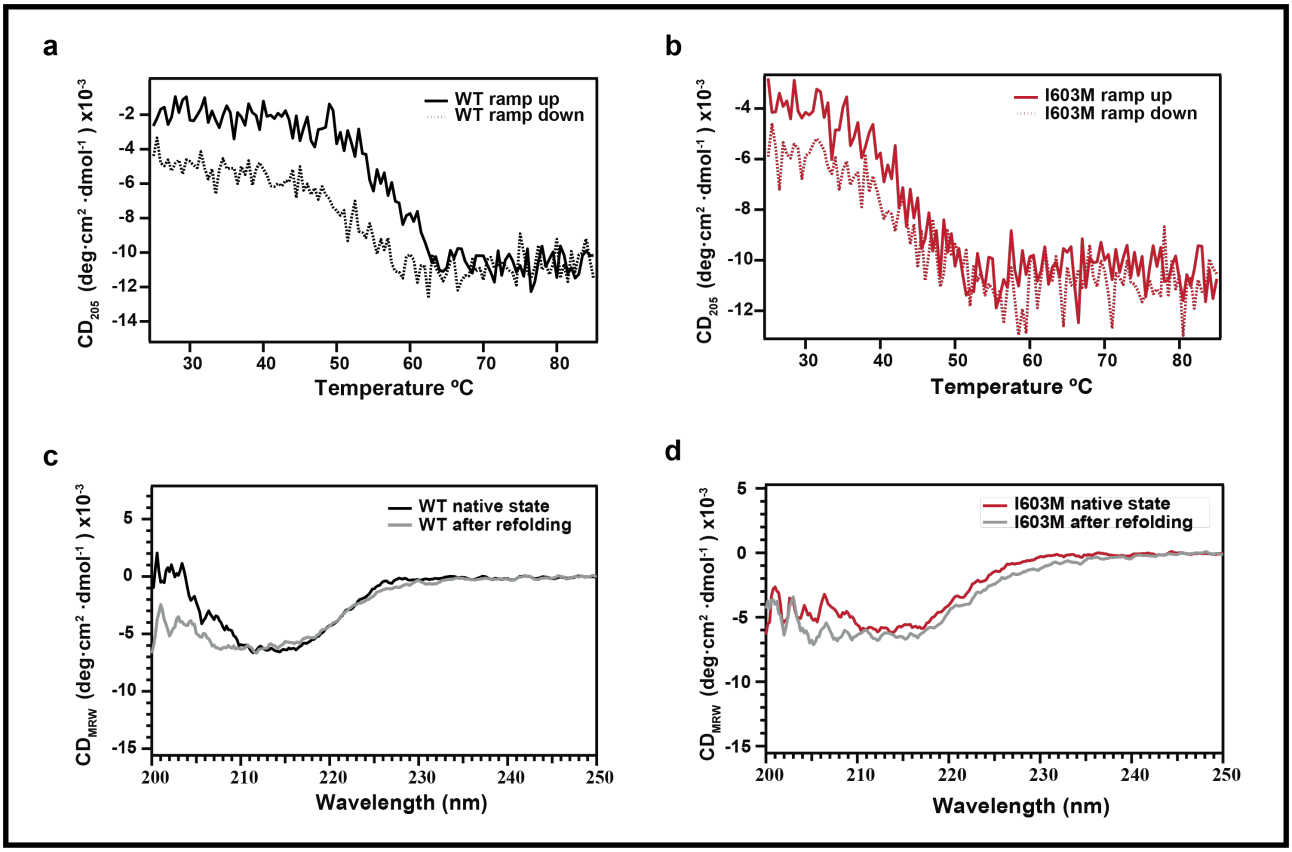


Supplementary figure 1: **Circular dichroism spectra of cMyBPC C4 WT and I603M before and after thermal unfolding.** CD spectra of C4 WT (a) and C4 I603M (b) monitored in the far-UV at 25°C (*solid line*) and 85°C (*dashed line*). Change in the shape of the far-UV spectra was observed when the sample temperature was increased to 85°C, indicating the unfolding of protein and loss of secondary structure for both WT and I603M. The maximum differences between spectra at 25°C and 85°C were observed at 230 nm. This wavelength was chosen for tracking the thermal denaturation of the proteins.



Supplementary figure 2: **Characterization of the reversibility of the thermal denaturation of the C4 domain.** Thermal denaturation (*solid line*) and renaturation (*dashed line*) of C4 WT (a) and C4 I603M (b) collected as change of ellipticity during thermal ramp up (from 25°C to 85°) and thermal ramp down (from 85°C to 25°C). The far-UV CD spectra of C4 WT (c) and C4 I603M (d) of protein before and after (*grey traces*) thermal denaturation/renaturation protocols.