

**S6 Table. Minimal activity and the activity amplitude of the actin/myosin S1-ATPase, measured in an enzyme coupled assay at pCa 9.8 and 4.5 as described before [14].** Briefly, reconstituted thin filaments containing wildtype cTnI, R170G or R170W were mixed with myosin S1 in presence of cMyBP-C. The ATP hydrolysis rate of actin/myosin S1 ATPase was measured by detecting inorganic phosphate release via an enzymatic cleavage of MESG by purine nucleoside phosphorylase. Data are given as activity  $\pm$ SEM (normalized to the activity of unregulated F-actin). P values from Student's t-test vs. cTnI-WT are given. n is the number of measurements.

cTnI	$y_{\min}$	P( $y_{\min}$ ) vs. WT	Amp	P(Amp) vs. WT	n
WT	0.43 $\pm$ 0.08	–	0.88 $\pm$ 0.09	–	9
R170G	0.37 $\pm$ 0.06	0.557	0.74 $\pm$ 0.08	0.262	9
R170W	0.64 $\pm$ 0.06	0.058	0.61 $\pm$ 0.09	0.052	8