α-tropomyosin with D175N or E180G mutation in only one chain differs from tropomyosin with mutations in both chains.

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ONLINE SUPPORTING MATERIAL

Purification of WT-E180G-Tm heterodimer

Elution profile of WT-E180G-Tm heterodimer is shown in Fig. S1 in order to show the purification of the heterodimer. The process was the same as that for the D175N heterodimer described in the main text. Additionally the purity of the heterodimer is shown in Fig. S2. This shows that the purified heterodimer runs as a single band when crosslinked and as two bands of equal density when reduced.

Further analysis of Tm thermal transients shown in Fig. 3 A-D in the main text

The data from unfolding transitions in Fig. 3 were smoothed and differentiated (Fig. S3, grey line) with the best fit to Gaussian peaks superimposed (dotted line). The data derived from each fit are given in Table S1 including the fractional peak areas (a measure of the free energy change associated with each unfolding "domain"), the width of half peaks (measure of unfolding cooperativity) and the midpoints of unfolding. All the measured data after differentiation and fitting procedure showed three distinct peaks normally interpreted to represent unfolding domains but the interpretation of such domains is not clear for a linear coiled coil such as Tm.

In Fig. S3 *A* the data for D175N-Tm homodimers is shown since the data for WT (previously published in Kremneva et al.[1] and WT-D175N-Tm heterodimer were all identical. In Fig. S1, *A*, *C* and *E* the data in the absence of DTT are shown fitted to three distinct peaks which are similar in all cases. The major peak is at ~57.1 -

57.4 °C and remains a similar size for all dimers. The middle peak is at 49 - 52 °C and is small (8-10 %) for the WT and D175N-Tm dimers but increases in area to 20-30 % for the E180G-Tm dimers. The lowest temperature peak shows the biggest change from 35 - 36 °C for WT and D175N to 30.1 °C for the WT-E180G-Tm heterodimer and to 26.4 °C for the E180G-Tm homodimer. This is a large change and is well below normal body temperature for these E180G dimers.

For the WT and D175N mutations the isotherms after loss of the crosslink (Fig. S1 *B* and Table S1) show a characteristic decrease in stability for the domains at high temperature (from 57 and 49 °C to 53 and 47 °C) with an apparent increase in stability for the low temperature domain from 35 - 36 °C to 40 - 41 °C accompanying an increase in area from 20 % to 30 – 35 %. Three similar peaks are seen for the E180G-Tm homodimer but the lowest transition again occurring at ~ 4 °C lower temperature (36 °C) and now being the largest peak at ~ 40 % of the total area. The broad nature of this peak means that a large fraction of this domain will be unfolded at 37 °C.

The fitted peak midpoints for the WT, WT-D175N and D175N-Tm in reduced conditions, shown in Table S1, have similar values however the measured isotherm of the WT-Tm (Fig. 3 *B*) showed ~1 °C shift from D175N-Tm constructs. The fitting process would suggest that this is primarily due to changes in the area of the peaks rather than a change in the peak position but the precision of the fits is not high.

Tm dimers	**Oxidised Tm (-DTT)**			**Reduced Tm (+DTT)**		
	Fractional Peak area (%)	Width of half peak (°C)	Peak midpoint (°C)	Fractional Peak area (%)	Width of half peak (°C)	Peak midpoint (°C)
WT-Tm	26.2 ± 0.5 14.4 ± 1.5 59.3 ± 1.3	10.1 ± 0.2 6.8 ± 0.4 7.3 ± 0.1	35.7 ± 0.1 49.1 ± 0.3 57.4 ± 0.1	44.7 ± 4.2 31.7 ± 4.7 23.6 ± 4.2	14.8 ± 0.9 5.6 ± 0.3 6.0 ± 0.4	41.2 ± 0.7 47.5 ± 0.3 53.1 ± 0.5
WT-D175N-Tm	27.3 ± 0.5 11.2 ± 0.9 61.5 ± 0.8	10.7 ± 0.2 6.0 ± 0.3 7.8 ± 0.1	36.7 ± 0.1 48.8 ± 0.2 57.2 ± 0.2	38.0 ± 3.2 28.4 ± 3.4 33.6 ± 3.3	16.7 ± 1.0 5.2 ± 0.2 6.4 ± 0.3	40.0 ± 0.7 47.5 ± 0.2 53.0 ± 0.3
D175N-Tm	28.0 ± 0.5 10.1 ± 0.7 61.9 ± 0.7	10.8 ± 0.2 5.7 ± 0.3 7.5 ± 0.1	36.3 ± 0.1 48.9 ± 0.1 57.2 ± 0.04	37.6 ± 4.5 34.1 ± 11.4 28.3 ± 11.8	16.1 ± 1.2 6.0 ± 0.4 6.6 ± 0.8	41.3 ± 1.0 47.9 ± 0.6 52.7 ± 1.1
WT-E180G-Tm	24.7 ± 0.7 35.6 ± 7.5 39.7 ± 7.2	10.6 ± 0.3 9.3 ± 1.0 6.0 ± 0.3	30.1 ± 0.1 51.3 ± 1.0 57.1 ± 0.1	47.6 ± 1.9 19.8 ± 2.7 32.5 ± 1.9	14.1 ± 0.5 5.1 ± 0.3 5.9 ± 0.2	36.4 ± 0.3 45.7 ± 0.2 51.7 ± 0.2
E180G-Tm	22.9 ± 0.3 27.7 ± 5.0 49.4 ± 4.8	7.9 ± 0.1 9.0 ± 0.7 6.3 ± 0.1	26.4 ± 0.04 51.8 ± 0.8 57.4 ± 0.1	48.3± 8.0 19.2 ± 3.4 32.6 ± 3.9	15.4 ± 1.2 5.2 ± 0.3 6.2 ± 0.3	39.1 ± 1.3 42.6 ± 0.1 52.1 ± 0.1

Table S1 Thermal unfolding of recombinant αTm. Data from the Gaussian fits shown in Fig S1. The fractional peak areas represent a measure of the free energy transition and are expressed in % of the total. The width of half peaks and the peak midpoints are given in °C. All the data are shown with their respective standard

errors and represents an average of at least two measurements of two independent samples ($n \geq 2$).

FIGURE S1

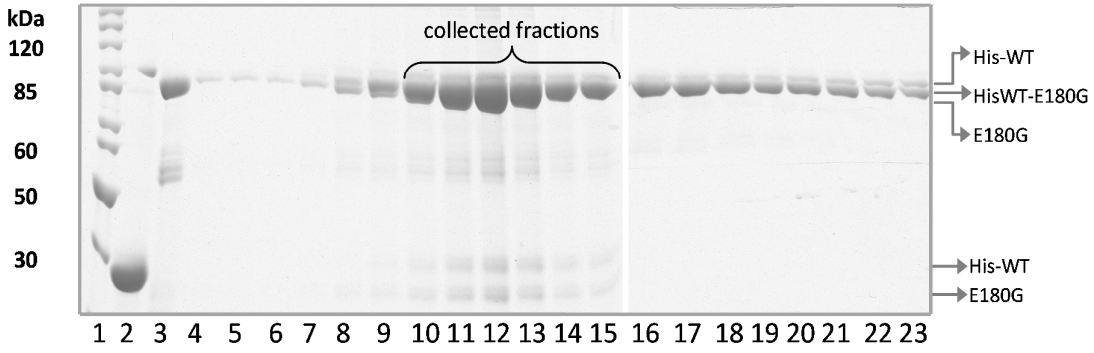

FIGURE S1 Evaluation of the affinity purification of α His-tagged WT-E180G-Tm heterodimers. Lane 1, MW marker; lane 2, control mixture of His-Tm homodimers and monomers; lane 3, control E180G-Tm homodimers. Lane 4 - 23 fractions from the column elution. Lane 4 - 7, E180G-Tm homodimers; lane 8 – 9, mixture of both E180G-Tm homodimers and His-tagged WT-E180G-Tm heterodimers; lane 10 – 15, His-tagged WT-E180G-Tm heterodimers; lane 16 – 23, mixture of both His-tagged WT-E180G-Tm heterodimers and His-Tm homodimers . Samples were run on a 10 % SDS-PAGE gel under non-reducing conditions.

FIGURE S2

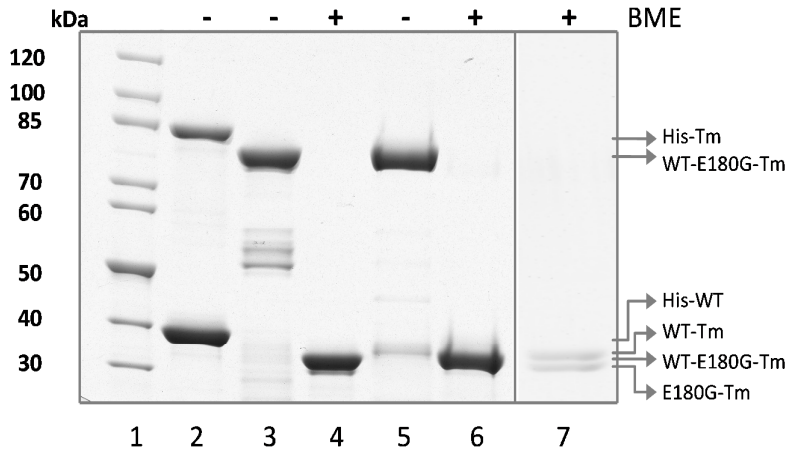

FIGURE S2 The isolation and purity check of WT-E180G-Tm heterodimers. Lane 1, MW marker; lane 2, mixture of His-Tm dimers and monomers; lane 3, E180G-Tm homodimers in reducing conditions; lane 4, E180G-Tm homodimers in non-reducing conditions; lane 5 & 6, assembled, crosslinked & purified WT-E180G-Tm heterodimer after removal of His-tag ,run under non-reducing (lane 5) and reducing conditions (lane 6) . Samples were run on a 4-12 % SDS-PAGE gel. Lane 7 shows WT-E180G-Tm heterodimer from this purification as used in cosedimentation assays, run on a 10 % gel to show better separation of the two different monomer chains. Note the expected 1 : 1 ratio of densities .

FIGURE S3

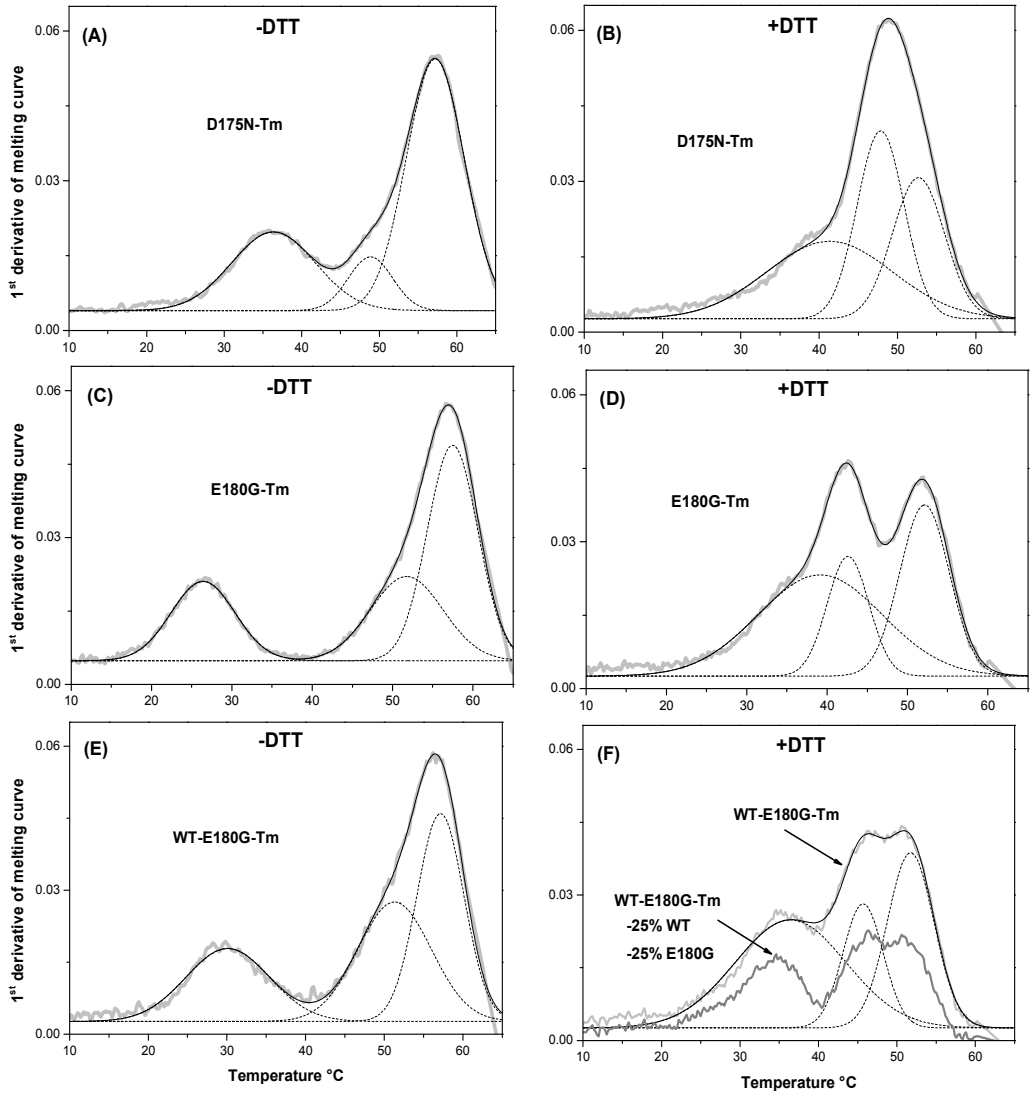

FIGURE S3 Thermal unfolding domains of skeletal αTm dimers with D175N and E180G HCM mutations. Each melting curve shown in Fig. 2 was smoothed (Savitzky-Golay method; 50 points of window), differentiated and then fitted to multiple Gaussian peaks. A summary of the fitted data is shown in Table S1.

References

1. Kremneva, E., Boussouf, S., Nikolaeva, O., Maytum, R., Geeves, M. A. & Levitsky, D. I. (2004). Effects of two familial hypertrophic cardiomyopathy mutations in alpha-tropomyosin, Asp175Asn and Glu180Gly, on the thermal unfolding of actin-bound tropomyosin. *Biophys J* **87**, 3922-3933.