Supplementary Materials for

The Z-disc protein α-actinin-2 forms a force-activated, directional bond with F-actin

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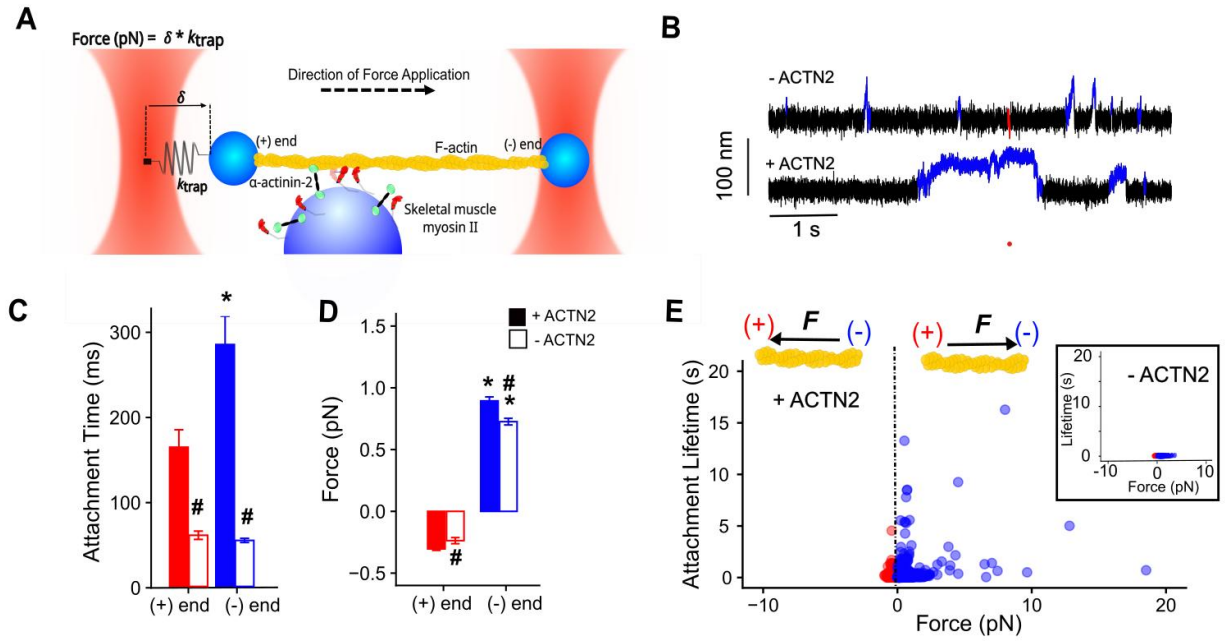

Fig. S1. Directional bonds by α-actinin-2 occur under (-) end directed force generation by skeletal muscle myosin. A) Optical trap assay. An actin filament is stretched between micron-sized beads held in optical traps. A third bead stuck to a coverslip is sparsely decorated with small ensembles of α-actinin-2, and in some experiments fast skeletal muscle myosin II. Myosin II moves along F-actin towards the (+) end, resulting in one of the two beads pulled out of the center of its trap, which reveals the orientation of the actin filament relative to the traps. Force equals the distance (δ) the bead is pulled from the center of the laser trap multiplied by the trap stiffness (k_{trap}). **B)** Raw displacement records. (+) ACTN2 signifies additional of α-actinin-2 to myosin ensembles. **C and D)** Mean ± SEM of attachment time and force. α-actinin-2 forms a directional catch bond with F-actin: binding lifetimes increase with force, but to a much greater degree when force is oriented toward the filament (-) end, **E)** Attachment lifetime vs force. Large plot shows + ACTN2 and insets shown skeletal muscle myosin II only. $p < 0.05$. Significant differences were detected using a non-parametric Kruskal-Wallis ANOVA using SPSS®. * Significantly different from (+)-end directed binding events. # Significantly different from + ACT2 condition. Sample sizes for – ACT2 were 433 events and + ACT2 were 1109 events. (+)-End and (-)-end directed events for -ACT2 condition were 64 and 369 events, respectively. (+)-End and (-) end directed events with the addition of α-actinin-2 (+ ACT2) were 239 and 870, respectively.

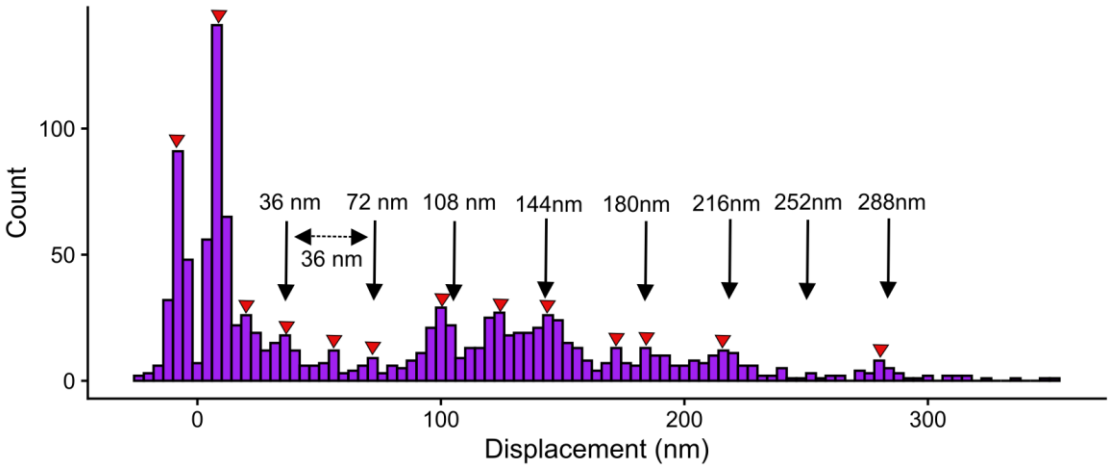

Fig. S2.α-actinin-2 binds along the periodicity of F-actin. Displacement distributions of WT ACTN2. Peaks within the distribution correspond with the 36 nm apparent periodicity and 72 nm helix periodicity of F-actin. This behavior is only exhibited under (-)-end directed force events.

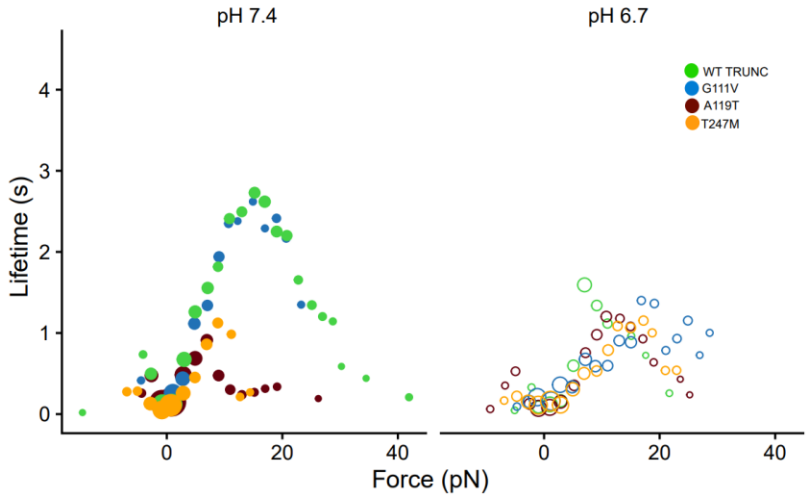

Fig. S3. HCM disrupts directional catch bond of α-actinin-2. Plots display force-lifetime catch bonds of WT TRUNC (green), G111V (blue), A119T (red), T247M (yellow) of α-actinin-2 constructs. Left panel shows catch bond at pH 7.4 and right panel shows catch bond at pH 6.7. See Table S1 for two-dimensional Kolmogorov-Smirnov comparisons.

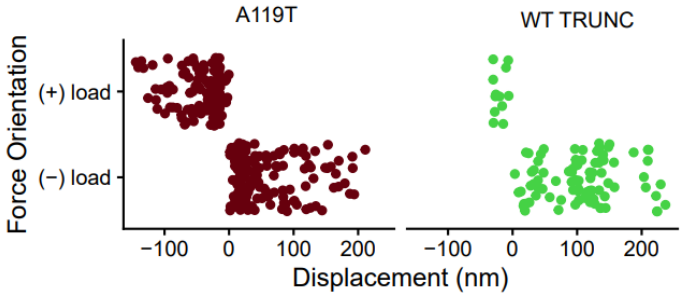

Fig. S4. A119T HCM mutation disrupts directional binding of Z-disc ensembles. A small number of α-actinin-2 molecules (~ 2 to 3 molecules) interact with an actin filament under (+) and (-) end directed force. $p = 0.043$ (A119T) and 1.2x10-13 (WT) for null hypothesis of equal number of binding events in both directions. $p < 0.001$ for null hypothesis that ratios are equal (two-proportion z-test).

Condition	A119T pH 6.7	A119T pH 7.4	G111V pH 6.7	G111V pH 7.4	T247M pH 6.7	T247M pH 7.4	WT pH 6.7	WT pH 7.4	WT TRUNC pH 6.7
WT TRUNC pH 7.4	0	5.64e-40	5.74e-28	1.06e-08	0	4.79e-37	6.89e-68	0	1.56e-12
A119T pH 6.7		0	0	0	0	0	0	0	0
A119T pH 7.4			4.89e-43	1.09e-22	0	5.96e-08	8.42e-132	0	2.356e-46
G111V pH 6.7				2.34e-12	0	5.16e-40	1.22e-93	0	6.00e-15
G111V pH 7.4					0	1.06e-26	9.59e-44	0	5.91e-12
T247M pH 6.7						0	0	0	0
T247M pH 7.4							4.82e-123	0	2.29e-46
WT pH 6.7								0	1.11e-34
WT pH 7.4									0

Table. S1. α-actinin-2 catch bond comparisons. Matrix of two-dimensional Kolmogorov-Smirnov test on all ACTN2 constructs. Calculated p -value is shown. Level of significance was $p < 0.05$ and a Bonferroni correction was applied. Zero values represent p-values too small for calculation.

pH 7.4

Condition	WT		WT TRUNC	
	Pearson's Chi-Square	Test of Conditional Independence	Pearson's Chi-Square	Test of Conditional Independence
WT TRUNC	0.58	0.086		
G111V	0.212	0.285	0.514	0.625
A119T	0.003	0.006	0.282	0.358
T247M	0.003	0.006	0.282	0.358

pH 6.7

Condition	WT		WT TRUNC	
	Pearson's Chi-Square	Test of Conditional Independence	Pearson's Chi-Square	Test of Conditional Independence
WT TRUNC	0.002	0.002		
G111V	<0.001	<0.001	0.015	0.24
A119T	<0.001	<0.001	<0.001	<0.001
T247M	< 0.001	<0.001	0.004	0.007

Table. S2. α-actinin-2 binding ratio comparisons. Matrix of two-proportion Z-tests on all ACTN2 constructs. All constructs at each pH showed significant differences ($p < 0.000$) for tests of homogeneity of odd ratios between (+) and (-) load binding ratios. Top table shows pH 7.4 and bottom table shows pH 6.7 results. Comparisons using Pearson Chi-Square and Mantel-Haenszel for tests of conditional independence for differences between constructs. Level of significance was set at $p < 0.05$ and values account for Bonferroni corrections.