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Supplemental Information

Dynamic Regulation of α-Actinin's Calponin Homology Domains on F-Actin

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Supporting Material

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Supplementary Information

A

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B

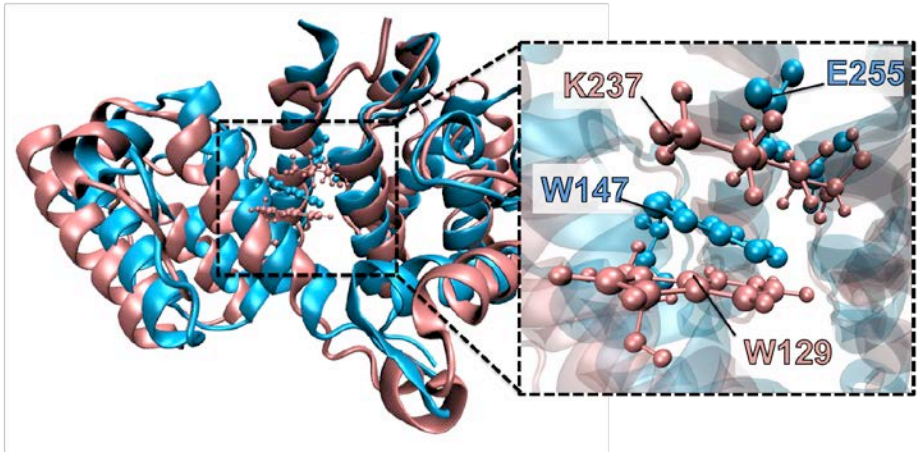


Figure S1. Sequence and structural alignments between human's K255E mutant α-actinin (*blue*, PDB ID: 2R0O) and chicken's wild type α-actinin (*pink*, PDB ID: 1SJJ). A) The sequence alignment between the chicken and human α-actinins shows that residue 47 of human's α-actinin maps onto residue 29 of chicken's α-actinin and therefore residue 255 of human's α-actinin aligns with residue 237 of chicken's. B) Structural alignment also shows that the position of K237 in the chicken ABD overlaps with residue E255 in the human α-actinin interacting closely with a nearby tryptophan W147, which corresponds to W129 in the chicken α-actinin.

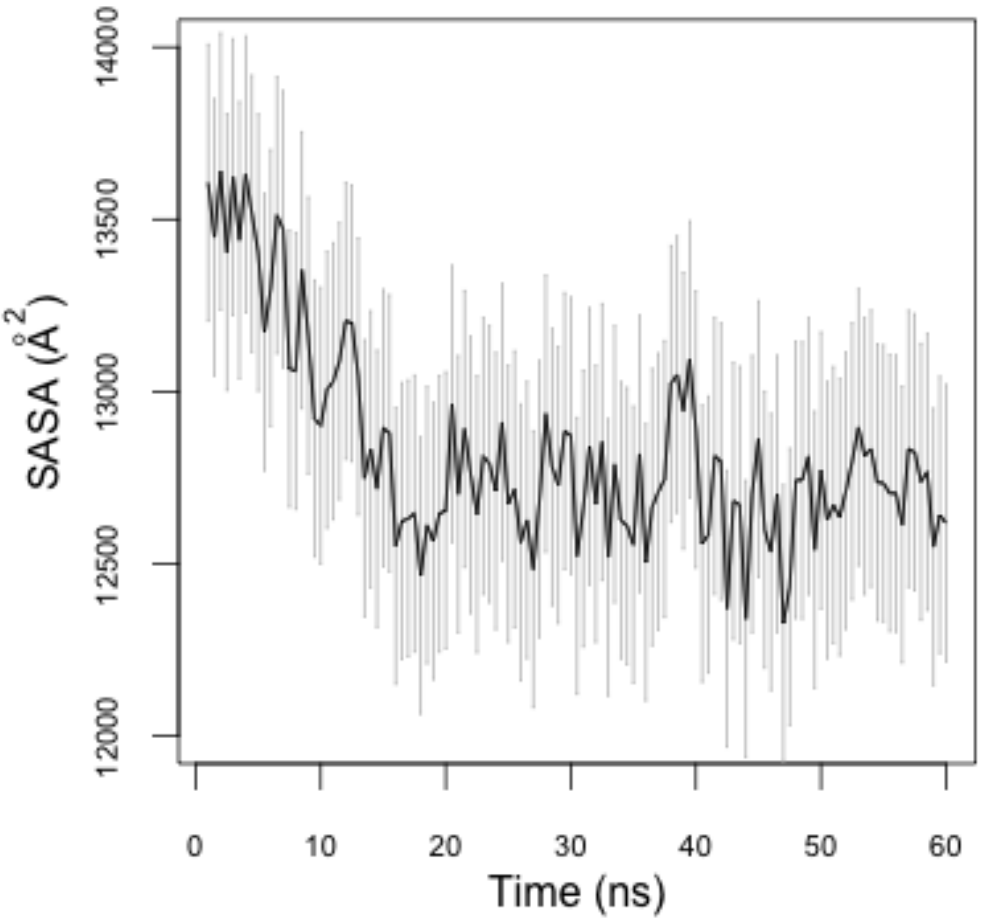
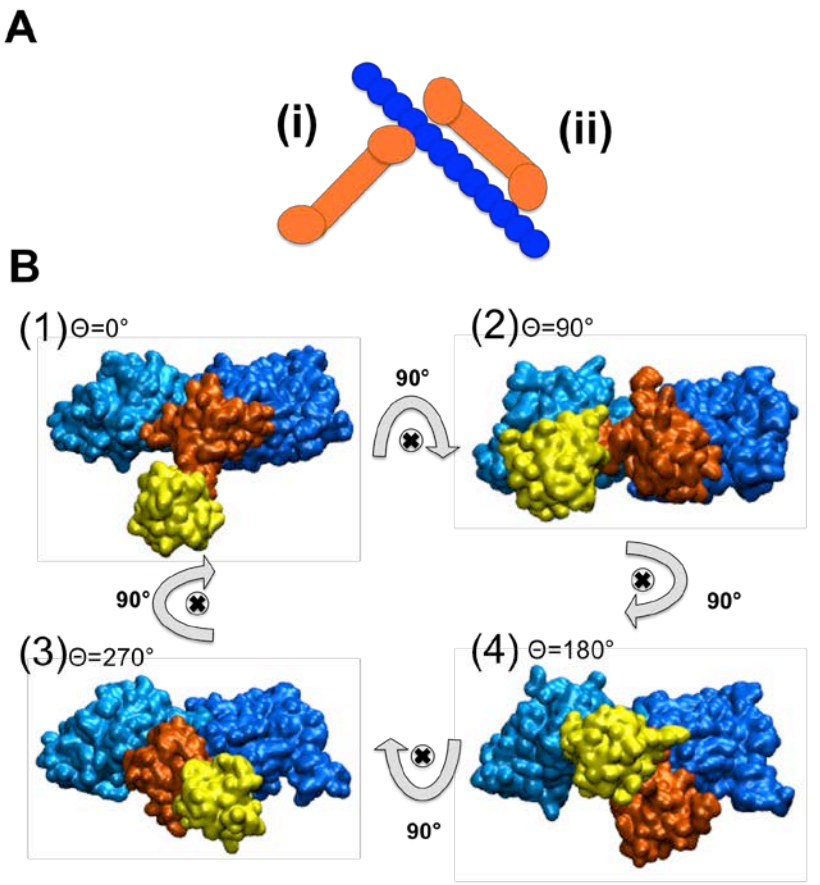


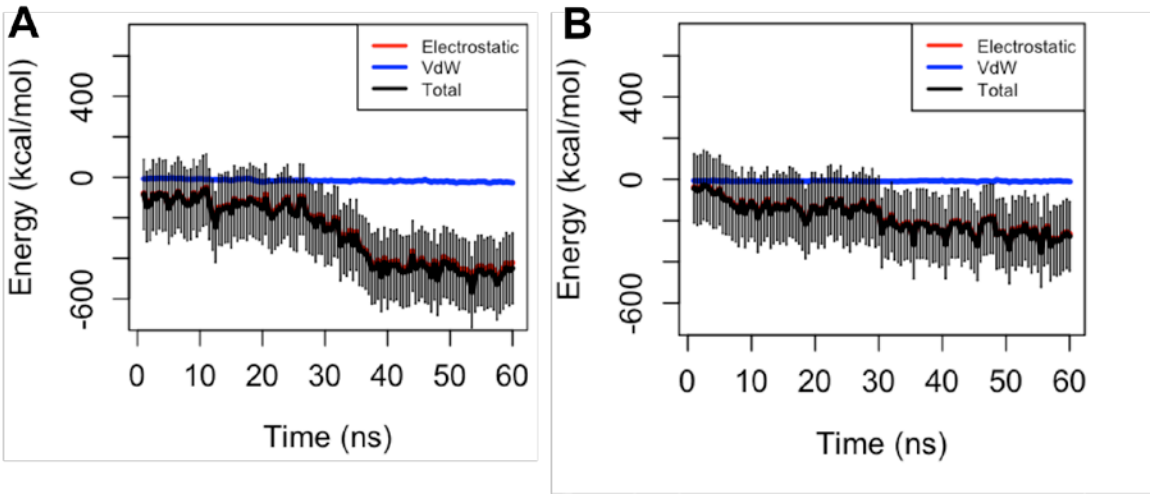
Figure S2. The average solvent accessible surface area (SASA) of CH2 in the CW simulations decreased in the first 20ns but remained constant afterwards.



C

	vdW	Electrostatic	Non-bonded
0	-3.13461	-16.0191	**-19.1537308**
90	-3.9473	14.59553	10.64823462
180	-10.739	-126.446	**-137.185023**
270	-9.89444	-90.7407	**-104.083758**

Figure S3. Different orientations of the oABD relative to actin were examined. A) α-Actinin can either (i) cross-link two actin filament or (ii) bind to a single one and thus more than one initial configuration might be favorable. B) The oABD was isolated from the rest of the α-actinin structure from which four different orientations were produced by rotating the oABD around an axis perpendicular to the F-actin axis relative to the orientation reported by Galkin et al. (reference 10 in the main text) (shown by ⊗). C) Comparing the binding energies of all oABD orientations to actin showed that the 180° rotated oABD formed the strongest binding in a shorter time (5 ns) compared to the original orientation (0°) used in all 60 ns binding simulations. Also, the 270° rotated oABD also formed a strong interaction after 5 ns.



S4. The interaction between CH1 and actin in A) the wild type and B) mutant α-actinins.

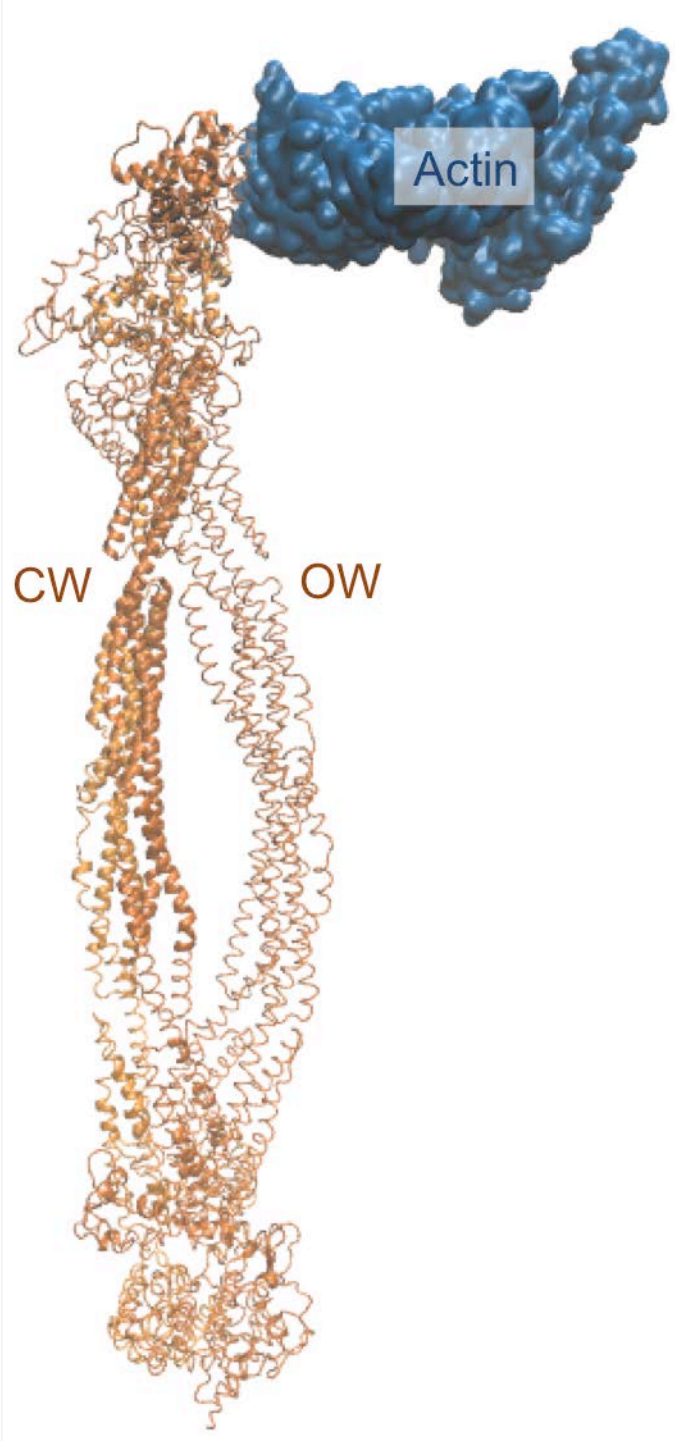


Figure S5. The curvature of the rod domain was different between the CW and OW simulations putting the ABD of the other end in slightly different positions.

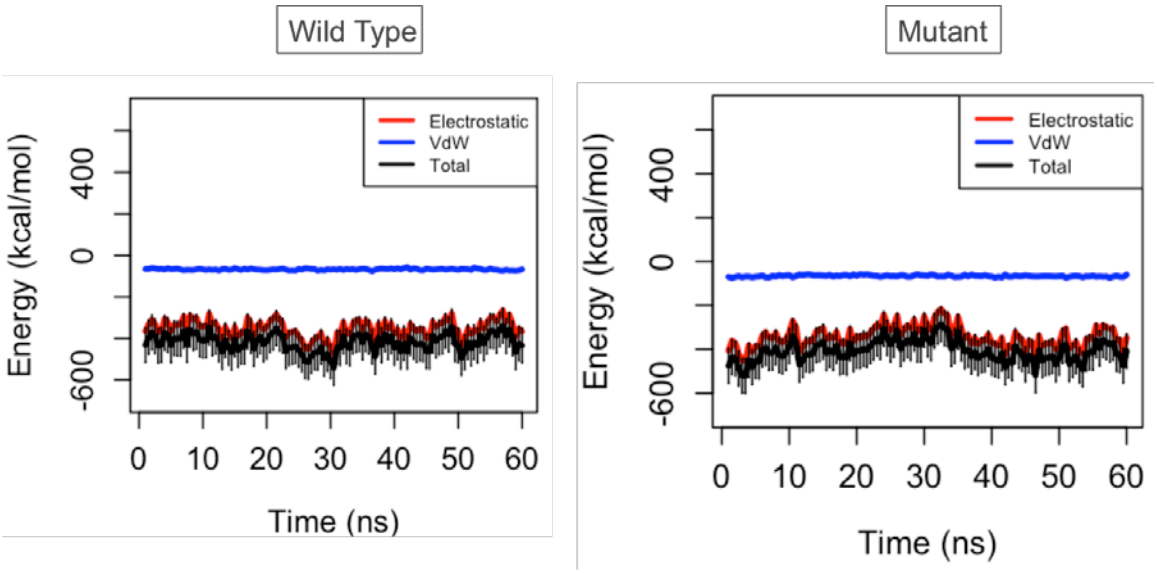


Figure S6. The CH1-CH2 binding energy averaged over three trials in the CW (*left*) and CM (*right*) simulations. The K237E mutation decreased the binding energy in the first 30 ns of the CM simulations but did not cause complete dissociation of the CH domains.

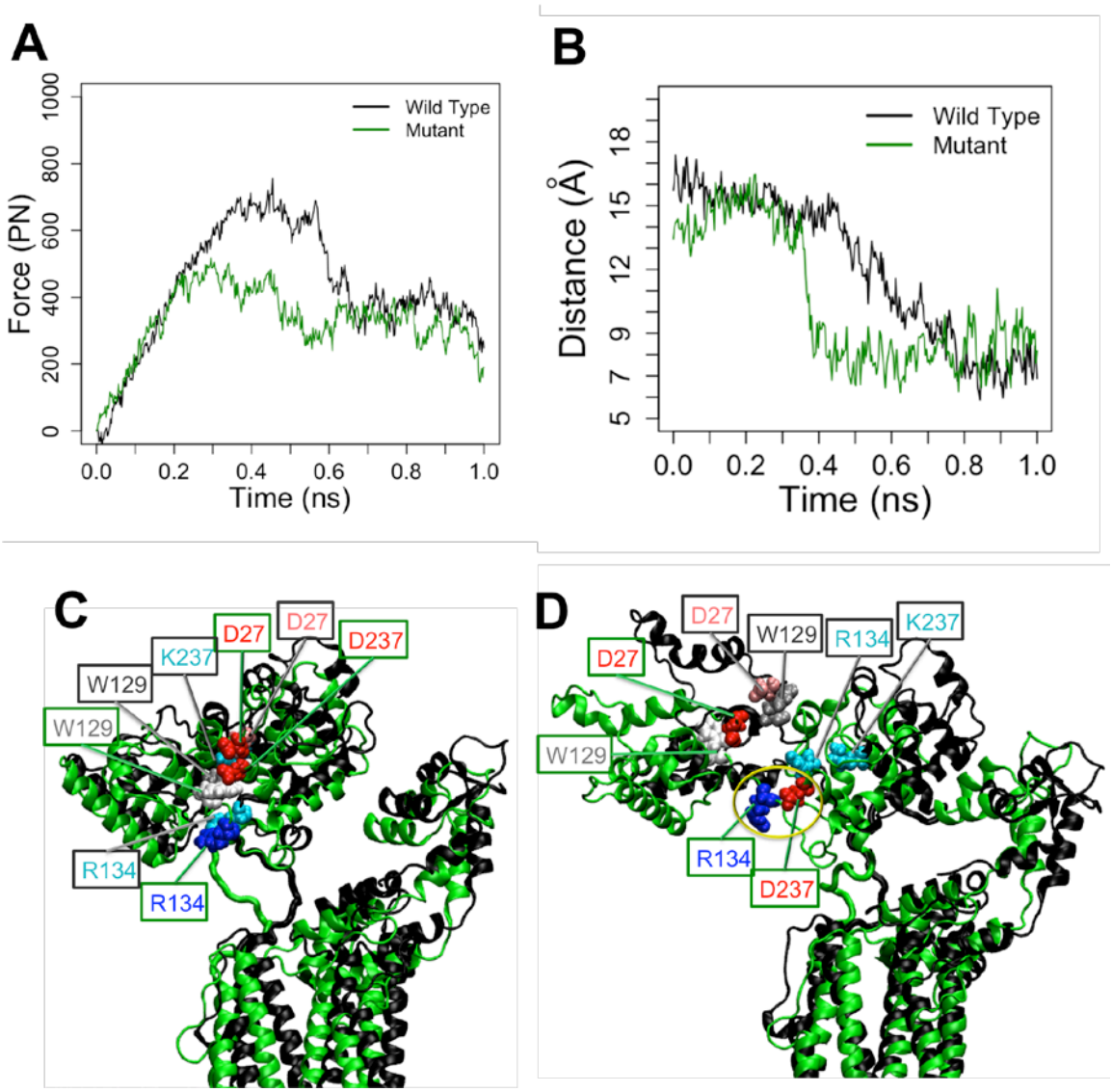


Figure S7. A) Forces used to separate the CH domains with a constant rate of 0.005 ps/nm for the wild type (black) and mutant (green) α-actinins. Clearly the mutant required lower forces for separating the CH domains. B) The distance between residues 237 and R134 in the neck region sharply changed in the mutant, which was simultaneous with the deviation in the pulling forces (A). C) The initial configuration of the wild type (black) and mutant (green) before pulling. All important residues are marked. D) After pulling, D237 and R134 formed an interaction that held CH2 close to the CH1-CH2 linker region that most likely prevented the reformation of the closed conformation.