SUPPORTING MATERIAL

Modeling the Assembly of the Multiple Domains of α-actinin-4 and its Role in Actin Crosslinking

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Running title: *On the core structure of multi-domain ACTN4*

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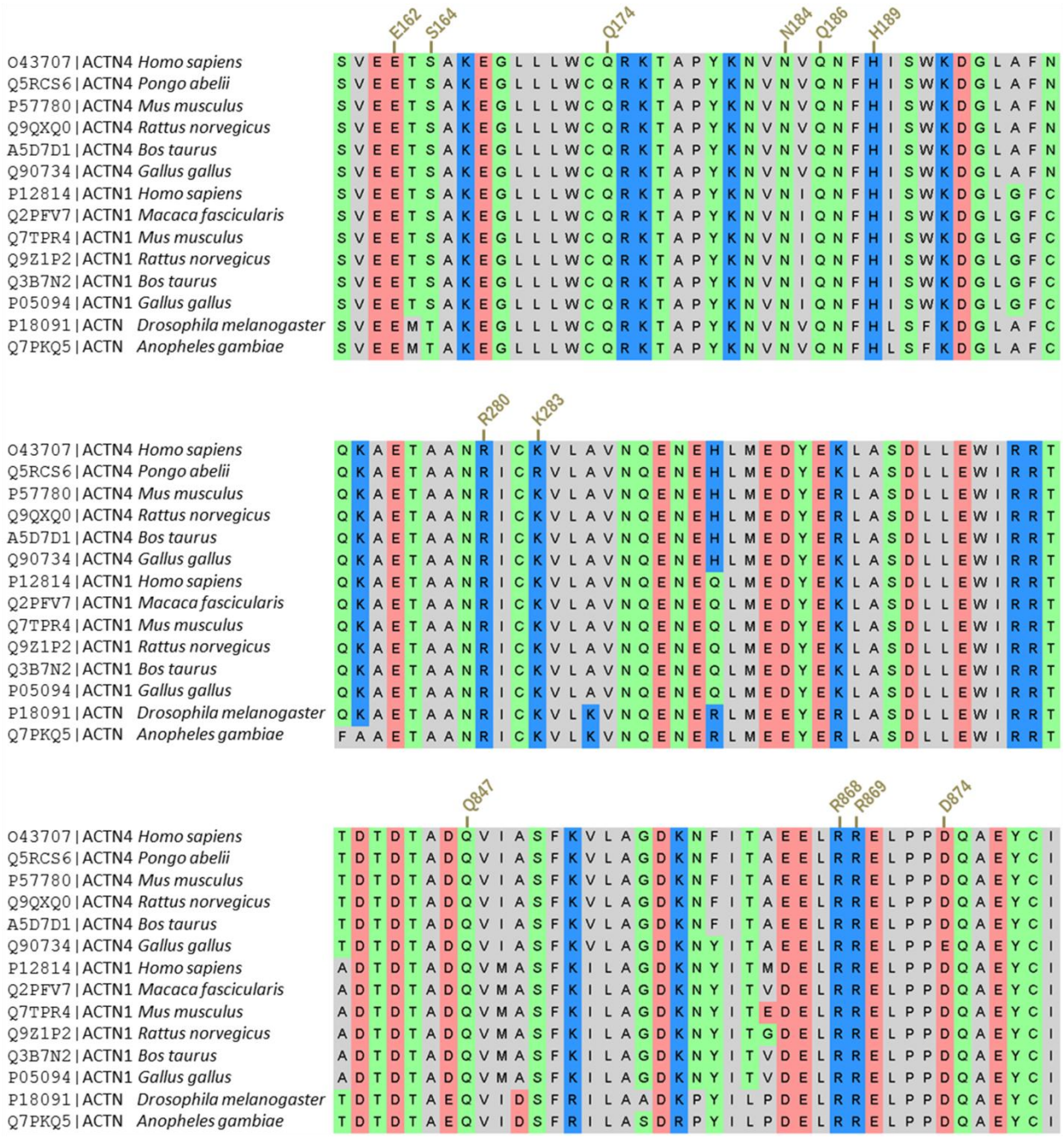

Figure S1. Residues that are predicted to stabilize the ABD-neck-CaM2 ternary complex are highly conserved among non-muscle ACTNs. Multiple sequence alignment (MSA) of non-muscle ACTNs is shown for only the regions around the contact residues (marked above each alignment, with numbering based on human ACTN4 sequence) validated in Figs.6–8. ACTN sequences are taken from the manually annotated (reviewed) section of UniProt (1); accession IDs are shown on the far left. Residues in the alignment are colored as follows: blue (positively-charged), pink (negatively, charged), green (polar), and gray (non-polar). MSA was generated using the implementation (with default parameters) of Clustal Omega (2) at the UniProt website.

Figure S2. Consensus secondary structure predictions show a disorder-to-order transition around Y31 for the Y4E/Y31E double phosphomimic mutant. Predictions of secondary structure were performed for (*A*) wild-type (WT) and (*B*) Y4E/Y31E mutant (MUT) sequences of the 45-residue ACTN4 N-terminal using the Network Protein Sequence Analysis (NPS@) web server (3). Consensus predictions are shown in bold for both sequences. The consensus prediction for the double mutant shows more consecutive residues adopting helical conformations around residue 31, consistent with the helicity plot shown in Fig. 10 *B*. Arrows point to the two mutation sites.

SUPPORTING REFERENCES

1. The UniProt Consortium. 2012. Reorganizing the protein space at the Universal Protein Resource (UniProt). Nucleic Acids Res. 40:D71-75.
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3. Combet, C., C. Blanchet, C. Geourjon, and G. Deléage. 2000. NPS@: network protein sequence analysis. Trends Biochem Sci. 25:147-150.