Supporting Material

"Tracking UNC-45 Chaperone-Myosin Interaction using a Titin Mechanical Reporter"

Christian M. Kaiser, Paul J. Bujalowski, Liang Ma, John Anderson, Henry F. Epstein, and Andres F. Oberhauser.

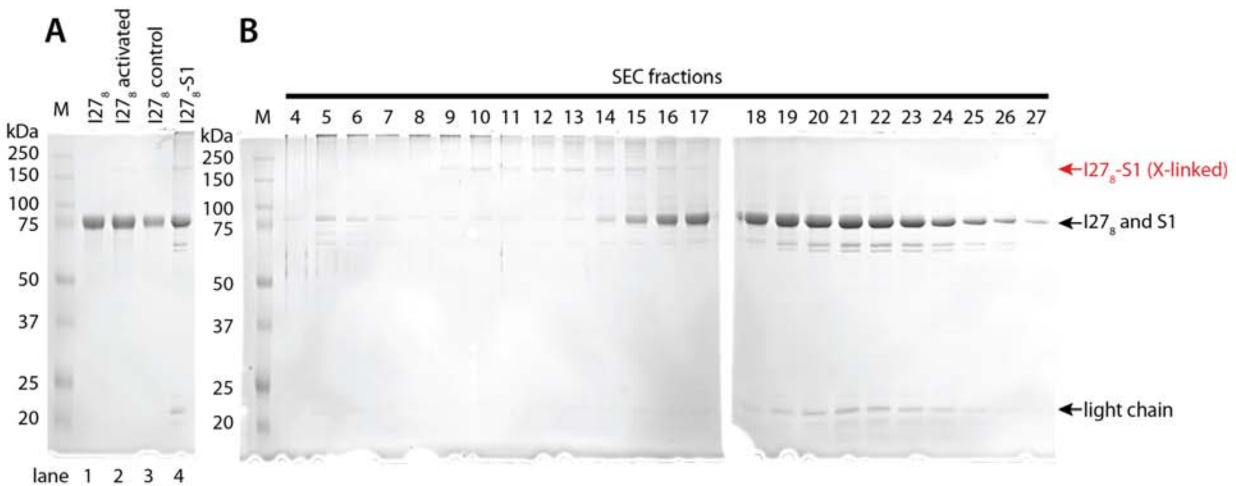

Figure S1. $I27_8$–S1 myosin crosslinking and product fractionation. A. SDS-PAGE analysis monitoring the progress of the $I27_8$-S1 coupling reaction. Lane 1: I27 octamer ($I27_8$, input); lane 2: activated $I27_8$ (after derivatization with the crosslinker $BM(PEG)_3$); lane 3: control reaction (incubation of activated $I27_8$ in the absence of myosin S1); lane 4: coupling reaction (incubation of activated $I27_8$ with myosin S1). The crosslinking product in lane 4 is apparent as a ~200 kDa band. B. SDS-PAGE analysis of crosslinking product fractionation by size exclusion chromatography (SEC) on a Sephacryl S300 column. The material shown in A (lane 4) was loaded onto the column and fractionated. Equal volumes of SEC fractions were loaded onto the SDS-PAGE gel for analysis. Due to its higher molecular weight, the crosslinking product elutes before the $I27_8$ and myosin S1 proteins. Fractions 11 to 13 were used for AFM experiments. $I27_8$ and S1 have very similar molecular weights and electrophoretic mobilities and are not separated on this gel. Note that the amount of $I27_8$ in lanes 3 and 4 is lower than in lanes 1 and 2 due to dilution resulting from the gel filtration step, and that the electrophoretic mobility of $I27_8$ and myosin S1 is indistinguishable under our electrophoretic conditions (lane 4). Gels in A and B were stained with Coomassie Brilliant Blue to visualize proteins. M: Molecular weight marker.

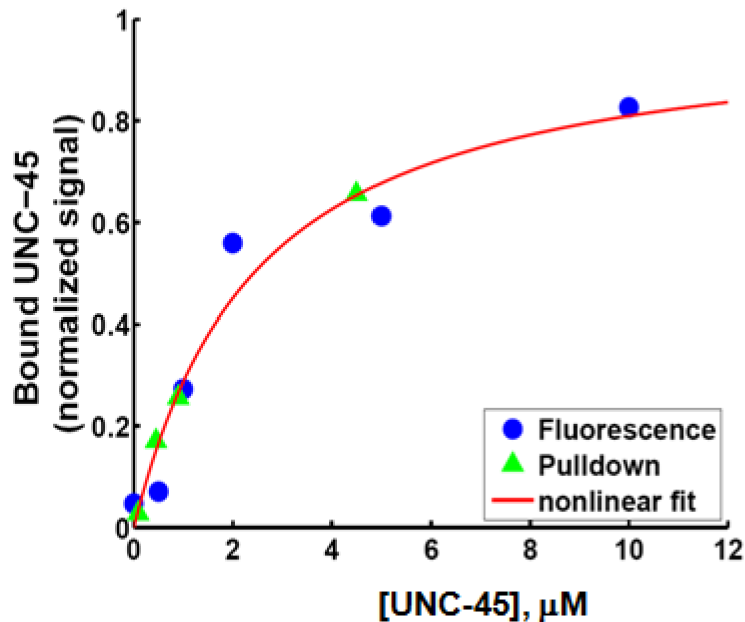

Figure S2. Normalized BADAN emission fluorescence (blue circles) and UNC-45b binding to myosin measured using a pull-down assay (green circles; from (1)) as a function of the UNC-45b concentration. The increase in fluorescence intensity reached a plateau at UNC-45b concentrations above 5 μM (blue circles). The green triangles in correspond to pull-down data obtained for UNC-45b binding to myosin (1). Both data sets follow a similar trend; by fitting the fluorescence and pull-down data assuming a simple ligand binding equilibrium, we obtained a dissociation constant K_d of 1.2 μM from. Both independent experimental techniques confirm that the recombinantly produced UNC-45b interacts robustly with the myosin motor domain.

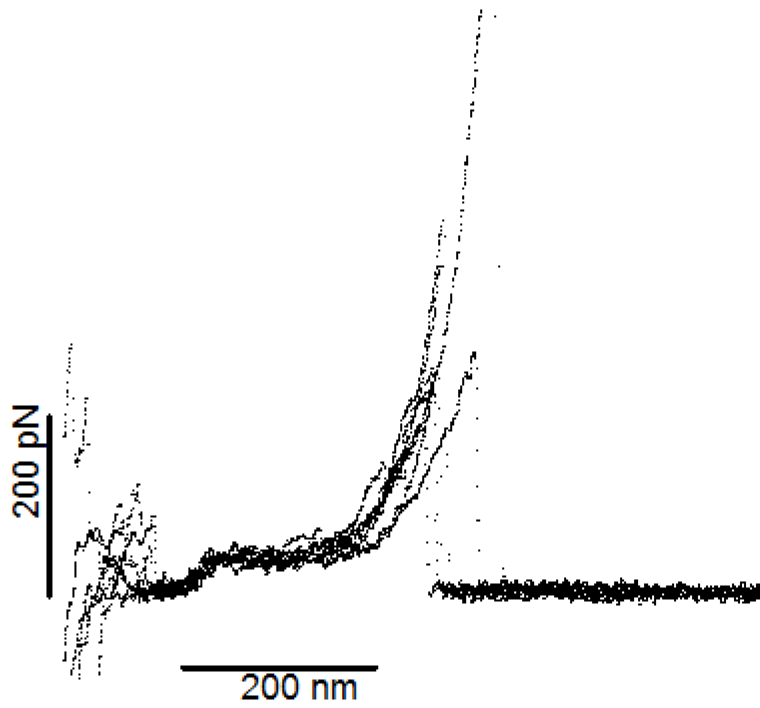

Figure S3. Force-extension curves obtained after stretching full length myosin purified from rabbit muscle. 8 different traces are shown.

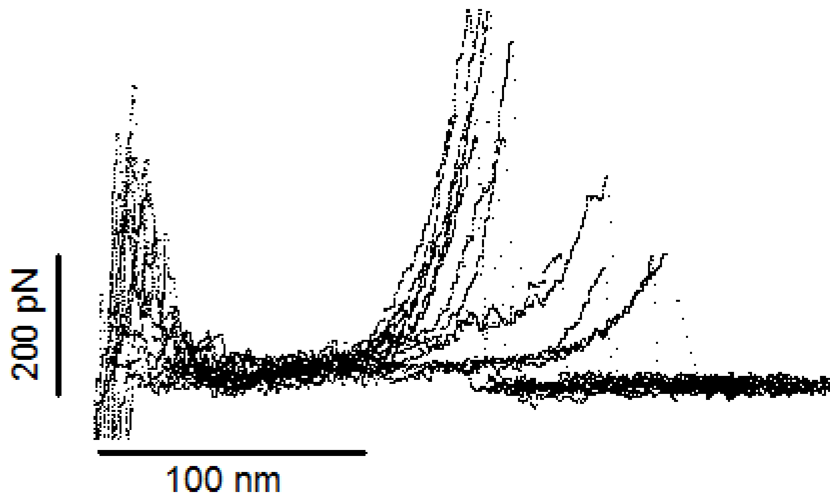

Figure S4. Force-extension curves obtained after stretching the S1 fragment. 11 different traces are shown.

References

1. Ni, W., A. H. Hutagalung, S. Li, and H. F. Epstein. 2011. The myosin-binding UCS domain but not the Hsp90-binding TPR domain of the UNC-45 chaperone is essential for function in Caenorhabditis elegans. Journal of cell science 124:3164-3173.