Supporting Material

The C2 domains of human Synaptotagmin 1 have distinct mechanical properties

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<u>**Supporting Methods**</u>

Single Protein Mechanics: Selection of 'bona-fide' recordings. The probability of pulling a polyprotein was kept very low (by adjusting the protein concentration). This was about ~5%, meaning that ~95% of force-extension (F-x) curves does not result in a contact with a molecule. The probability of pulling a polyprotein from end-to-end is even lower (0.1%). This is because of the random nature of attaching the AFM tip to the protein. Pulling a polyprotein like I27-I27- C2AB -I27-I27 should result in a sawtooth pattern with 6 force peaks, 4 coming from the I27 domains. However, because of the random attachment of the molecules, a substantial number of the traces are incomplete and contain mostly partial unfolding fingerprints. We have found that out of 10,000 F-x curves only 10 will have a full-length fingerprint.

In order to quickly screen for the effect of a large number of mutations we have developed an automatic AFM that collects a large number of force-extension curves (F-x) and automatically searches for recordings with the correct mechanical fingerprint coming from the unfolding of titin I27. Under this automatic mode, the AFM scans the surface tapping it constantly to collect thousands of F-x curves. The software also automatically corrects for sample and cantilever drifts. During 12hrs the machine typically collects ~10,000 F-x curves; these are analyzed off-line.

Some other representative force-extension curves for (I27)2-C2AB-(I27)2 are shown in <u>Supplemental Figure 1.</u> The data shown in Figures 2A and 4A do have some non-specific de-adhesion force peaks. However, for clarity we do not show these because it distracts from the main result. In supplement material Figure S1, we now show recordings with non-specific de-adhesion force peaks.
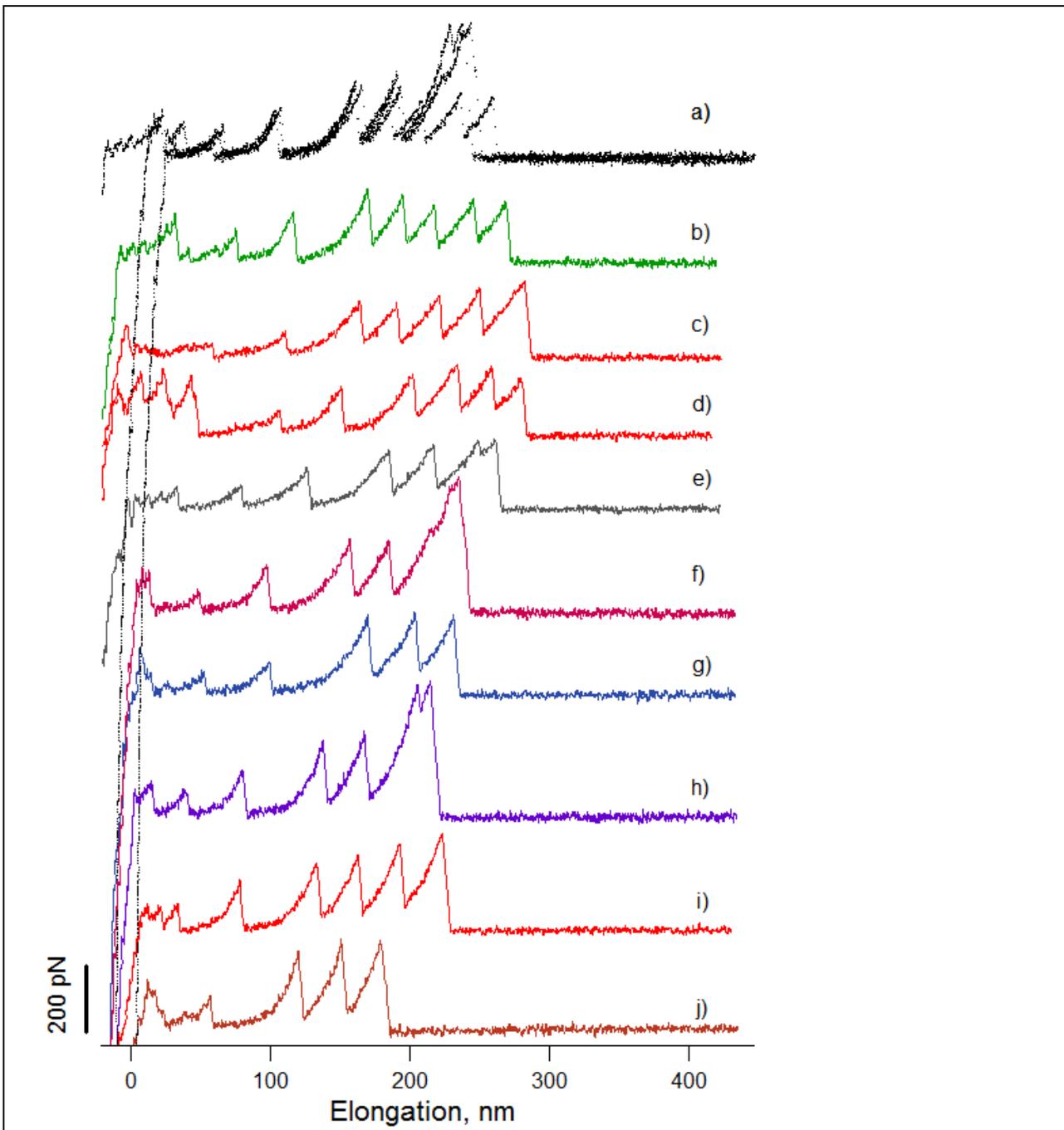
Supplemental Figure 1. Examples of force-extension curves obtained after stretching the (I27)2-C2AB-(I27)2. A) Superposition of many traces showing the unfolding pattern of C2 domains. The initial ~50nm have typically non-specific desorption peaks. B) More examples of stretching the (I27)2-C2AB-(I27)2 protein. 'a' is the superposition of three representative traces (displayed as dots). The initial ~30nm typically have non-specific desorption peaks that sometimes mask domain unfolding events. The initial two force peaks correspond to the unfolding of C2A and C2B, followed by the unfolding of I27 peaks. Traces 'b' and 'c' correspond to full extension traces, which capture both C2 domains and four I27 domains. Trace 'd' corresponds to a partial pull having both C2 domains and three I27 peaks. Trace 'e' through 'h' have both C2 domains and two I27 peaks. Trace 'i' and 'j' shows only C2B with three and two I27 peaks, respectively.