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Supplemental Information

Interaction of the Complexin Accessory Helix with Synaptobrevin Regulates Spontaneous Fusion

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Supporting Material

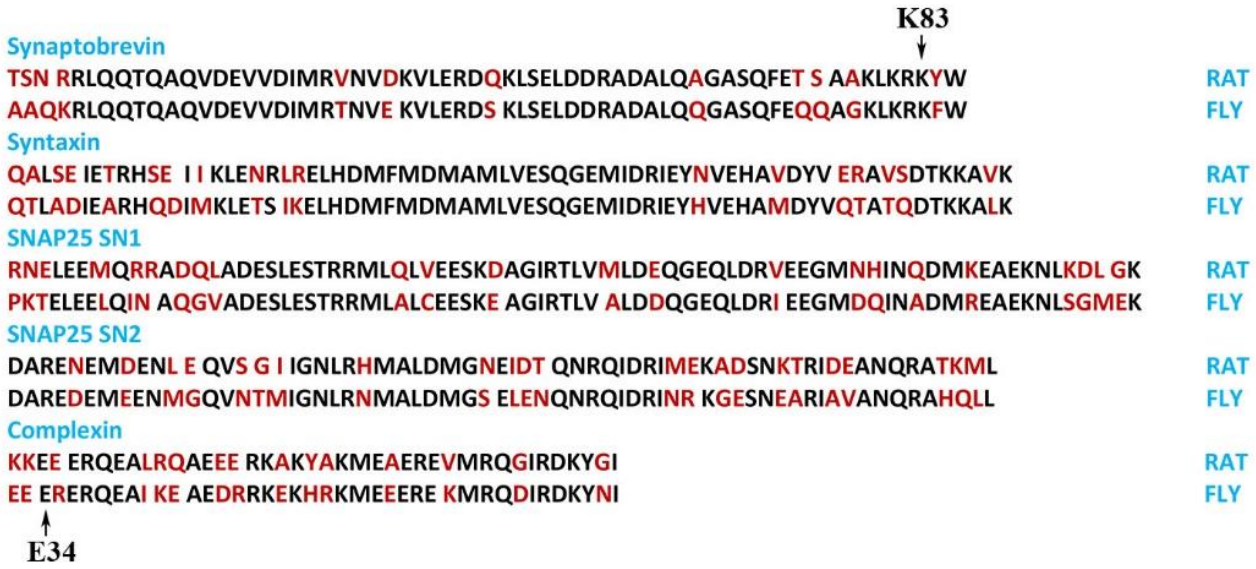

Figure S1. Homology between the mammalian and *Drosophila* SNARE proteins (substitutions are shown in red). The residues K83 of Syb and E34 of Cpx that mediate the interaction between Syb and Cpx are conserved.

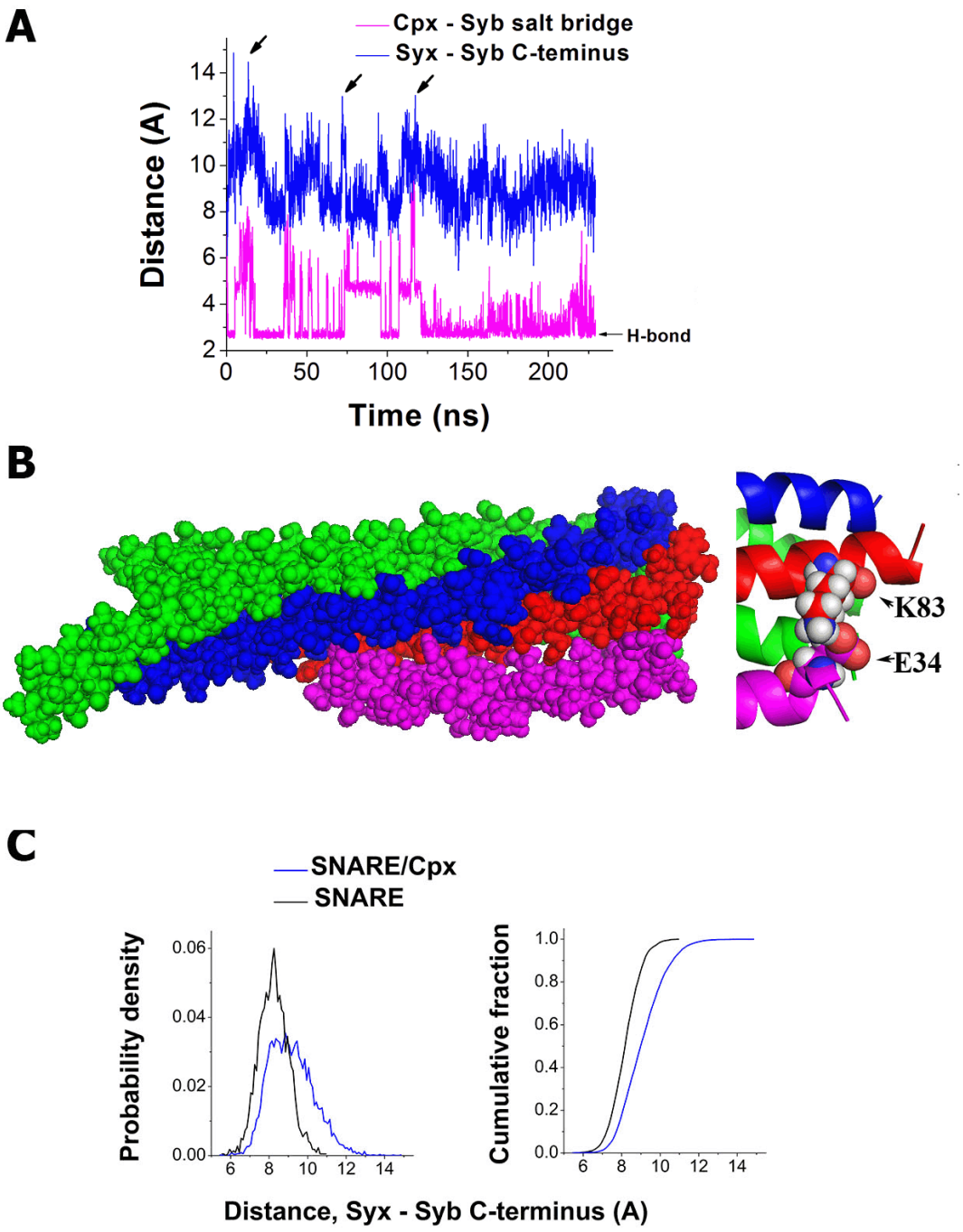

Figure S2. MD trajectory of the *Drosophila* SNARE-Cpx complex. **A.** Transients separations of Cpx from Syb, as well as a transient increase in the distance between the Syb and Syx C-termini (arrows) are shown. The distances are computed between Cα atoms of Syx and Syb C-terminal residues (blue), and the atoms defining the salt bridge between Syb and Cpx: Nz of Syb K83 and Oε of Cpx E34 (magenta). **B.** The most populated state of the SNARE bundle with the interaction between Syb and Cpx stabilized by a Syb K83 – Cpx E34 salt bridge. **C.** The distribution of the distances between C-terminal residues of Syb and Syx along the MD trajectories of the SNARE bundle and the SNARE-Cpx complex. The presence of Cpx shifts the distribution towards longer distances.

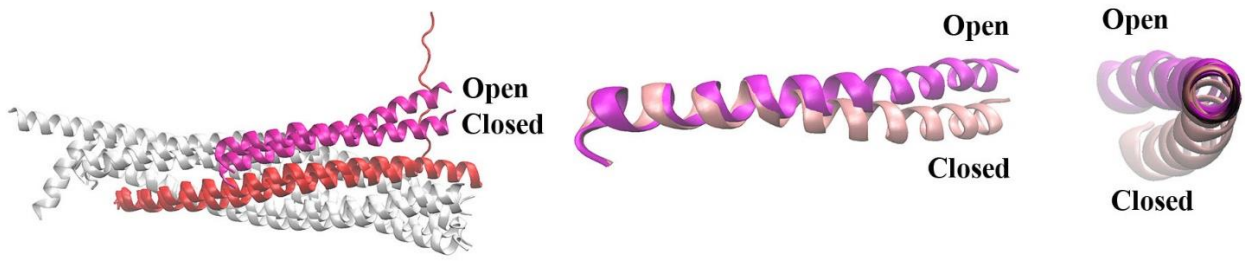

Figure S3. The closed state of Cpx produces a bending between the Cpx central and accessory helixes, and this kink may serve as a "spring" to accelerate Syb unraveling. Superimposed close and open states of the SNARE-Cpx complex are shown. Magenta: Cpx, red: Syb.

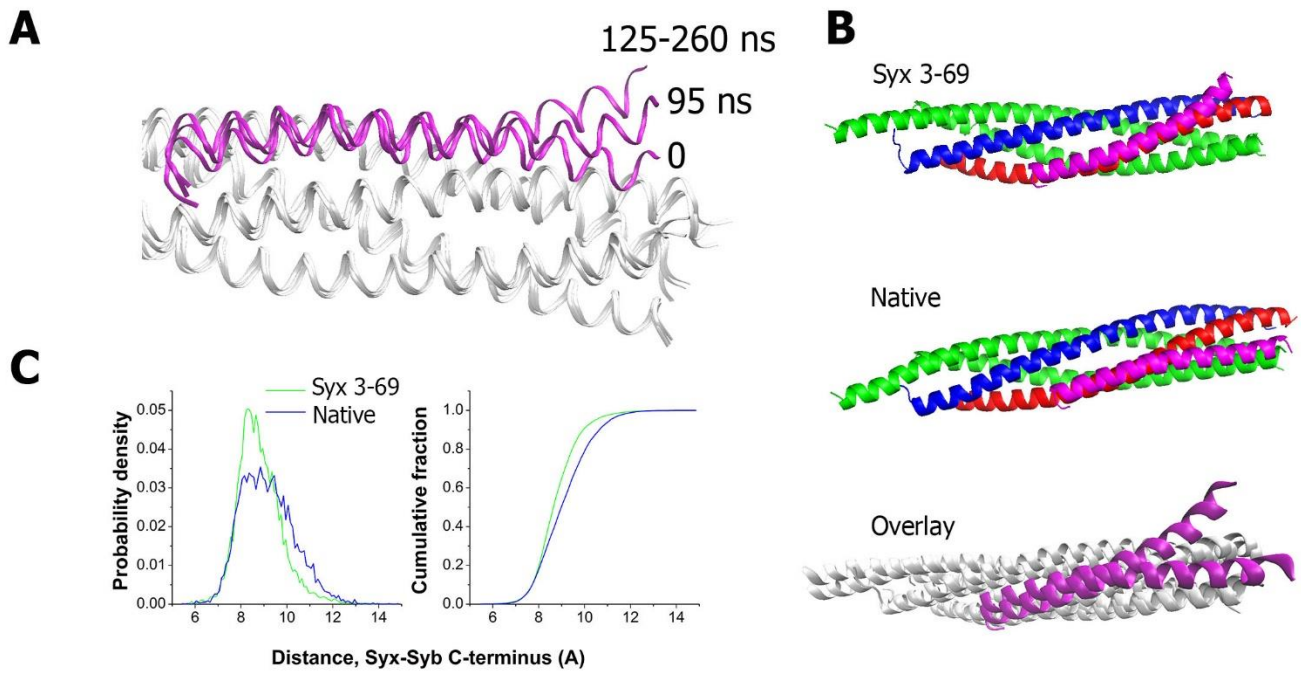

Figure S4. MD trajectory of the mutated (*syx*$^{3\text{-}69}$) SNARE-Cpx complex. A. Cpx (magenta) position on the bundle in the beginning of the MD trajectory (0), at an intermediate point (95 ns), and at the second half of the trajectory (125-260 ns). The SNARE proteins overlaid at these three points are shown in white. **B.** The *syx*$^{3\text{-}69}$ mutation changes Cpx conformation. **C.** The mutation shifts the separation between C-terminal residues of Syb and Syx towards shorter distances.

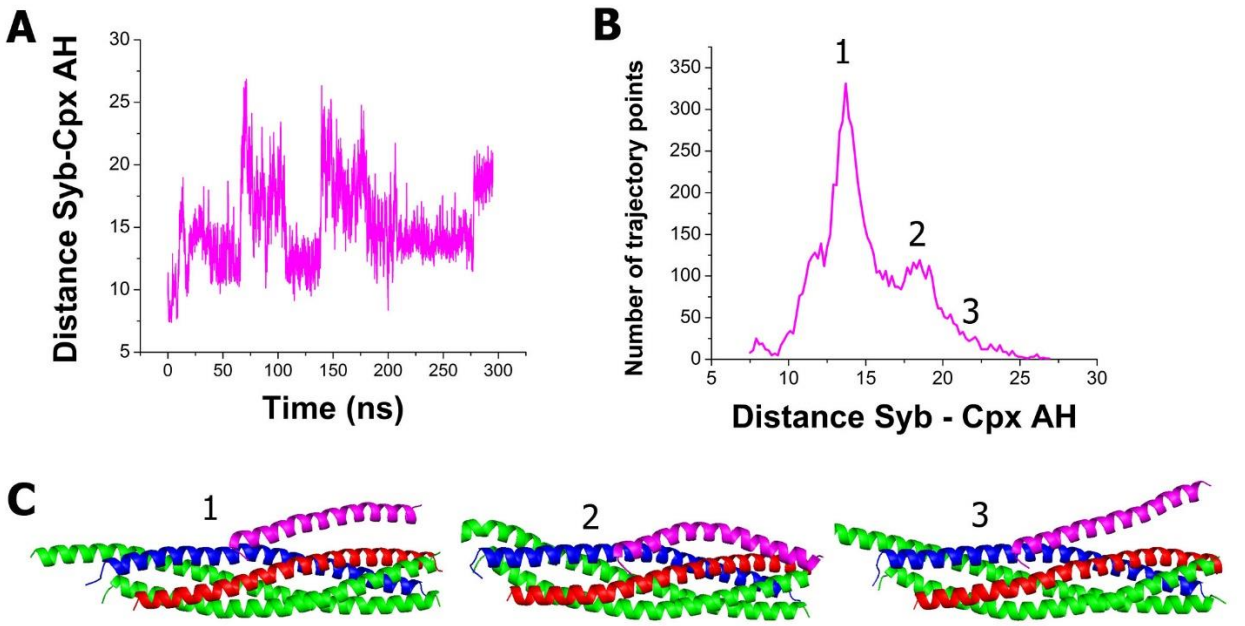

Figure S5. The MD trajectory of the SNARE-Cpx complex with the E34A Cpx mutation. **A.** The separation between Cpx AH and Syb. The distance is computed between Cα atoms of Cpx A34 and Syb K83. **B.** The frequency distribution of the distance between Cpx AH and Syb along the trajectory showing the states 1-3 (state 1 is the most populated). **C.** Three conformations corresponding to the three states in the panel B. State 1: Cpx lies parallel to the bundle; state 2: Cpx interacts with SN2 (green); state 3: open state of Cpx.

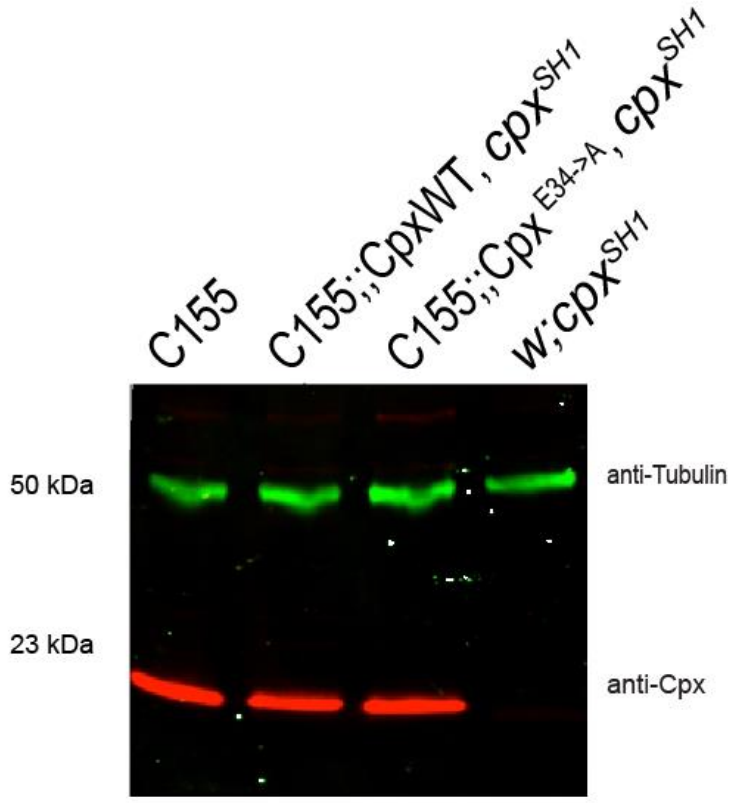

Figure S6. Western blot showing similar Cpx expression levels in the mutant line Cpx^{E34A}, cpx^{SH1} and the rescue line Cpx^{WT}, cpx^{SH1}. The cpx^{SH1} null mutant and the C155 driver line (elav-GAL4) are shown as controls. Anti-Tubulin antisera was used as a loading control.