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Supplemental information

Mechanical force can enhance c-Src kinase activity by impairing autoinhibition

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Supplementary information for:

Mechanical force can enhance c-Src kinase activity by impairing autoinhibition

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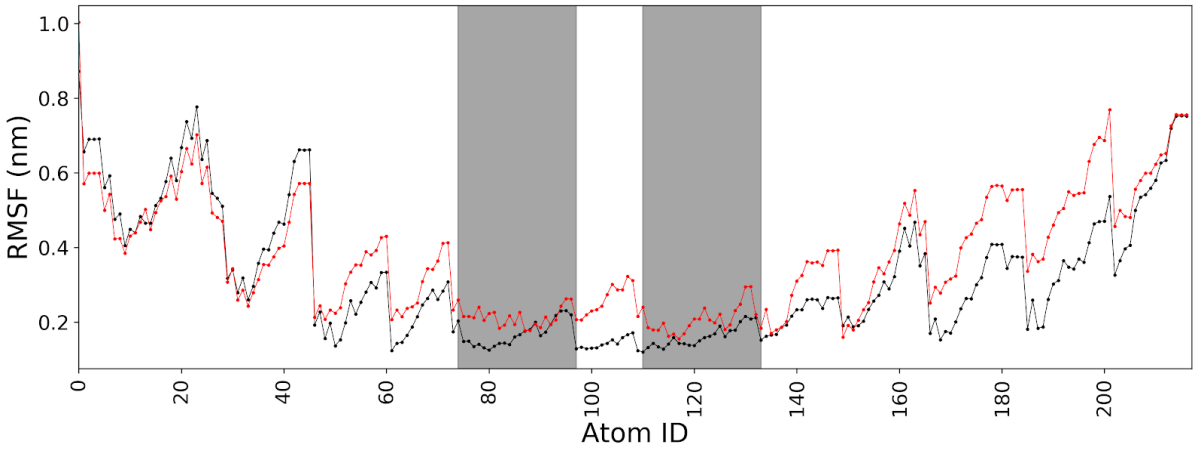

Fig S1: Root mean square fluctuations (RMSF) of the p130Cas peptide atoms while in complex with c-Src, with either the N-terminal (red) or C-terminal end (black) of the peptide oriented towards the C-terminal end of c-Src. The greyed areas of the graph encompass the atoms of the phosphotyrosine residues of the p130Cas peptides.

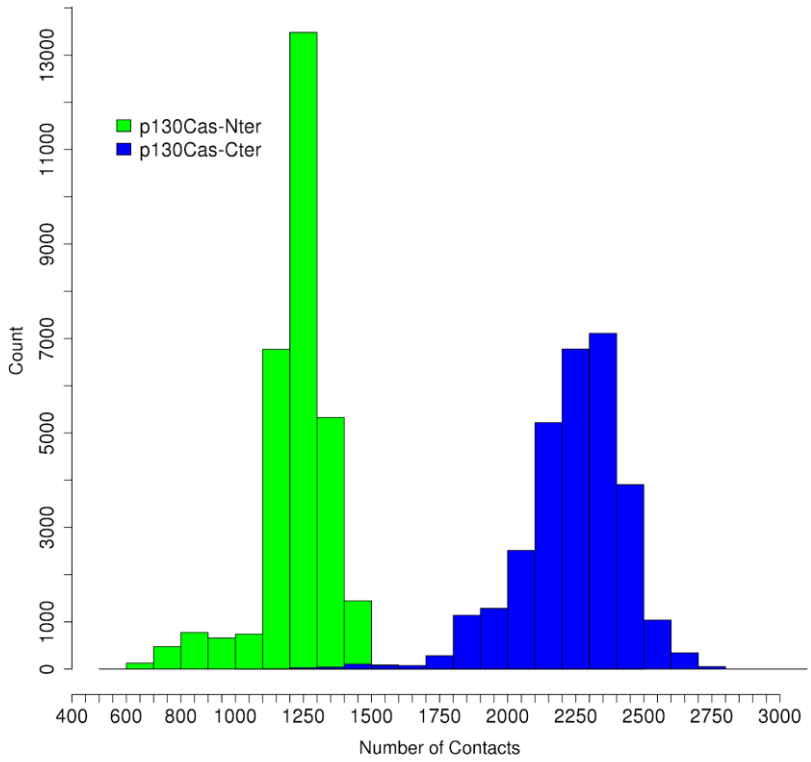

Fig S2: Stability of the p130Cas-cSrc complex with the peptide oriented with its N-terminal or C-terminal end towards the C-terminal end of c-Src. Number contacts between c-Src and the p130Cas peptide docked onto c-Src kinase domain with either its N-terminal (green) or C-terminal end (blue) towards the C-terminal end of c-Src.

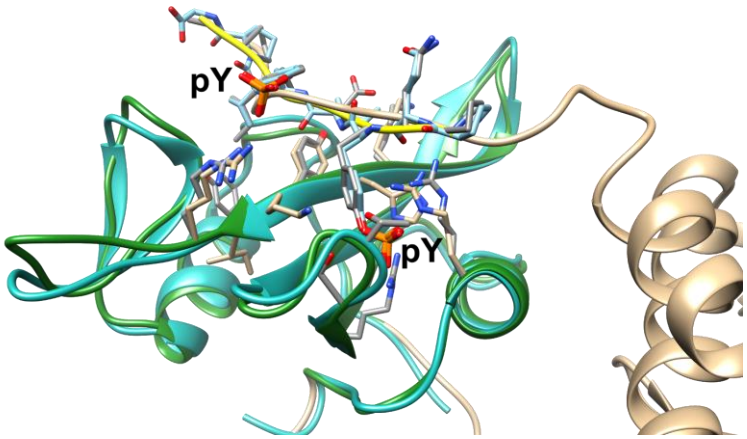

Fig S3: Modelling of the p130Cas in complex with c-Src. The figure shows the SH2 domains of c-Src, green, (PDB code: 2src) and Lck, cyan, (PDB code: 1x27) kinases. Part of c-Src kinase domain is coloured in beige, with its c-terminal end docked back onto the SH2 domain. The p130Cas peptide co-crystallised with the SH2 domain of Lck, and competing with the binding site of the c-terminal c-Src tail is shown in yellow. The overlap between basic residues of the SH2 domains, the p130Cas peptide and the c-Src c-terminal tail, with the atoms coloured by atom type, shows that the phosphotyrosine residues (pY) of p130Cas dock into a pocket composed of positively charged residues in the SH2 domain.

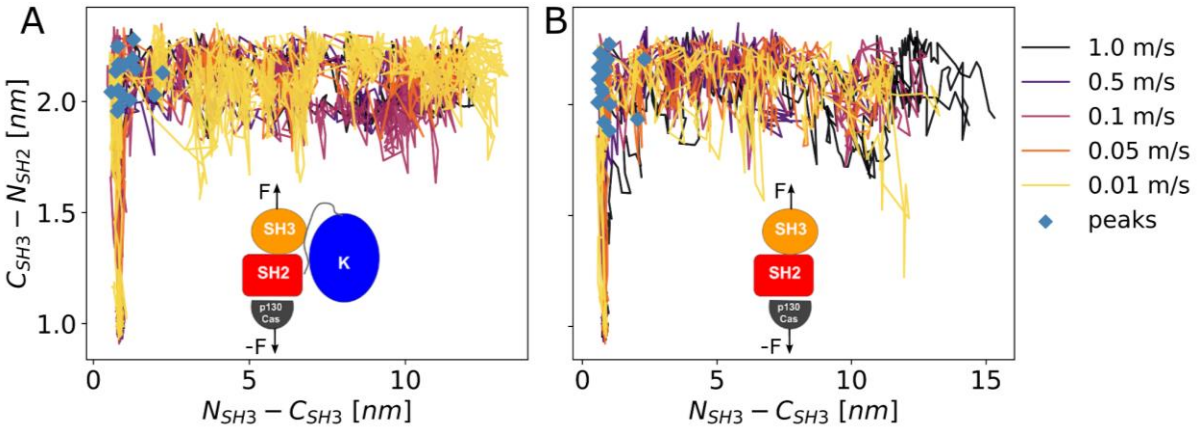

Fig S4: The kinase domain does not qualitatively affect c-Src force response. The unfolding behavior with (A) and without (B) the kinase domain is very similar: The SH3-SH2 linker extends first before the SH3 domain unfolds. The highest force peak (shown in blue) generally corresponds to the switch between these two stretchings. Compare to Fig 2 in the main text (showing panel B here).

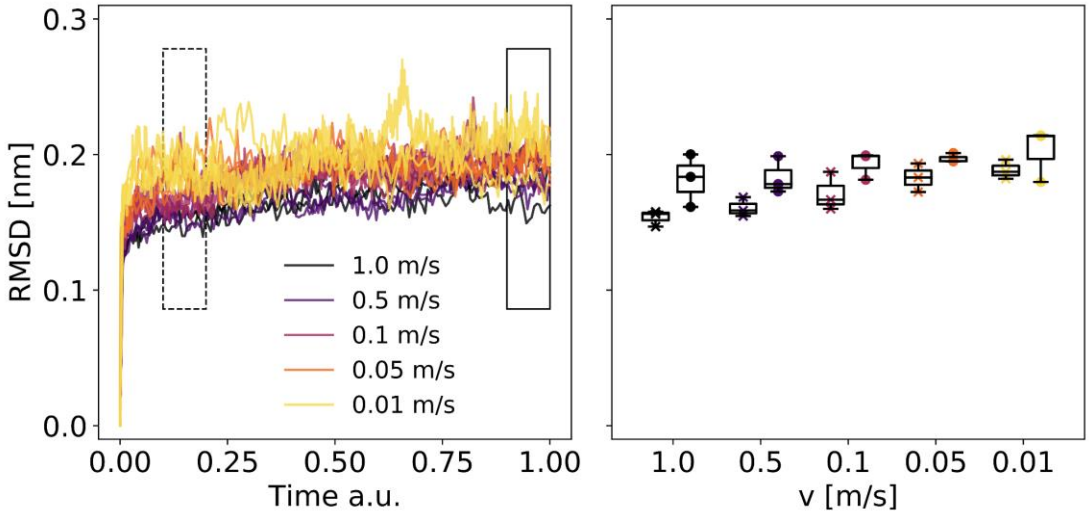

Fig S5: The kinase domain remains stable during force probe MD simulations. The RMSD of the whole kinase domain of c-Src (residues 267-518) is shown with time 0 denoting the start and 1 the end of each simulation. The small increase in average RMSD for each pulling velocity of less than 0.03 nm, which is observed when comparing the RMSD in a window at the beginning of the simulations before SH3 rupture (dotted outline, crosses) to the end (solid outline, circles), is expected considering that the kinase domain loses contact to its inhibitory domains.

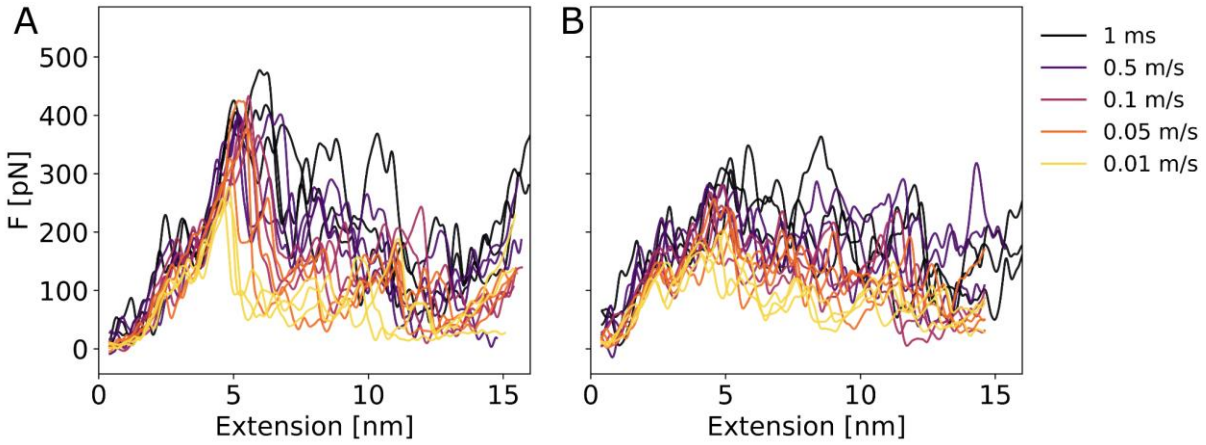

Fig S6: Force profiles with kinase (A) and without (B) as a function of extension (pulling speed times simulation time).

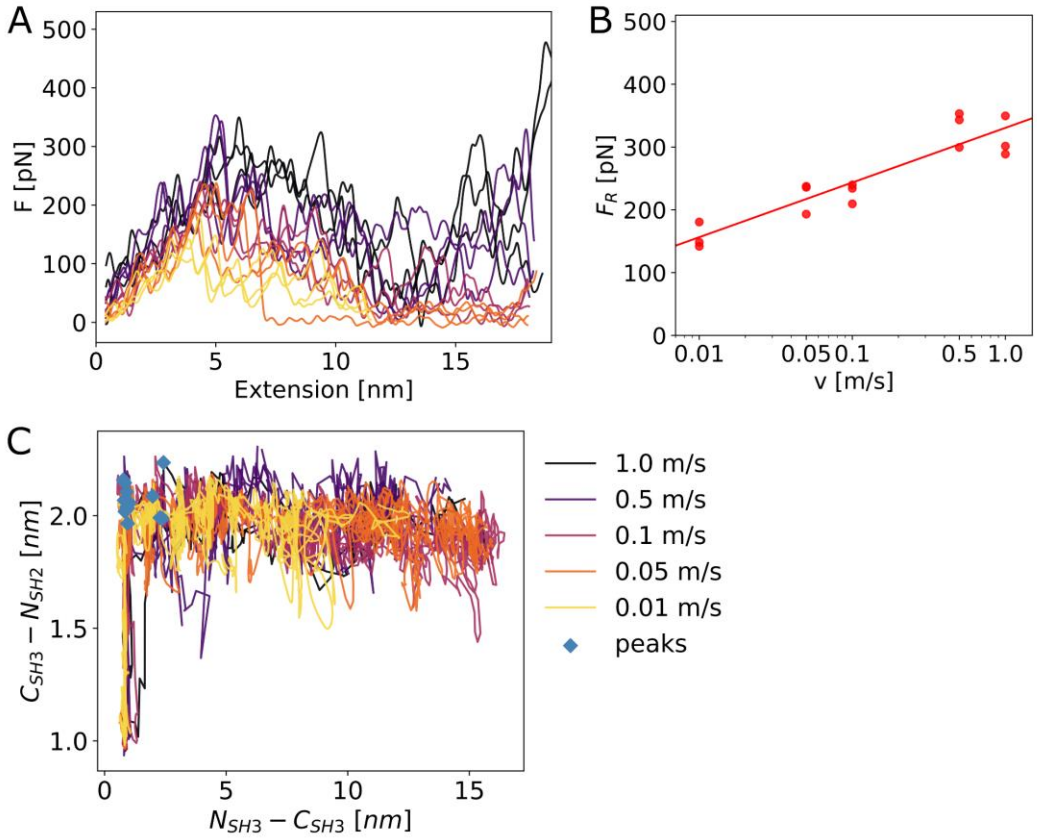

Fig S7: Force probe MD simulations of c-Src without kinase domain using the CHARMM36 force field. In comparison to simulations with the Amber99sb*-ILDN force field the force-extension profile (A, compare to Fig. S6B), rupture forces (B, compare to Fig. 3D) as well as the order of SH3-SH2 detachment and SH3 domain unfolding (C, compare to Fig. 2) are similar.

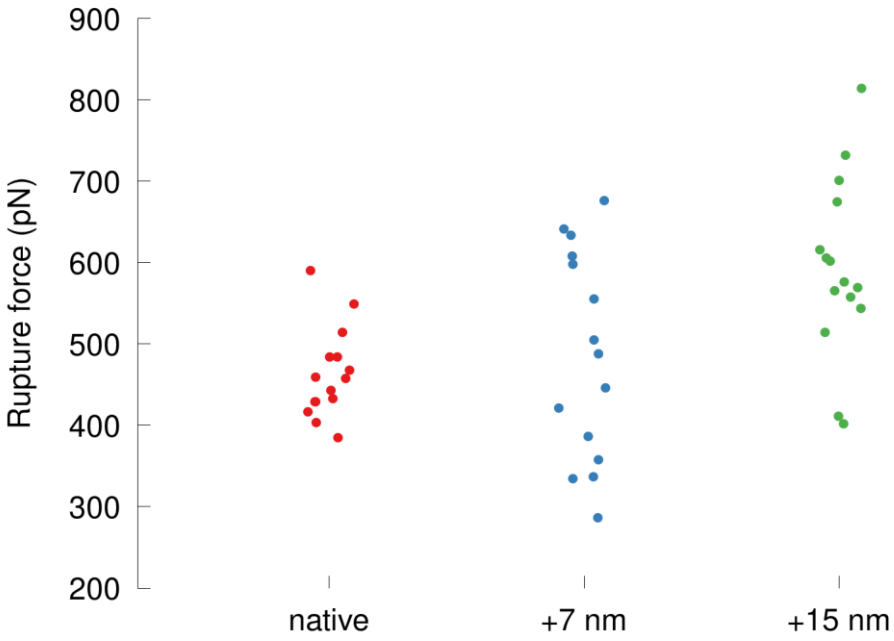

Fig S8: Rupture forces for enforced activation are not lower at non-native structures. We show 15 rupture forces, one for each initial frame.

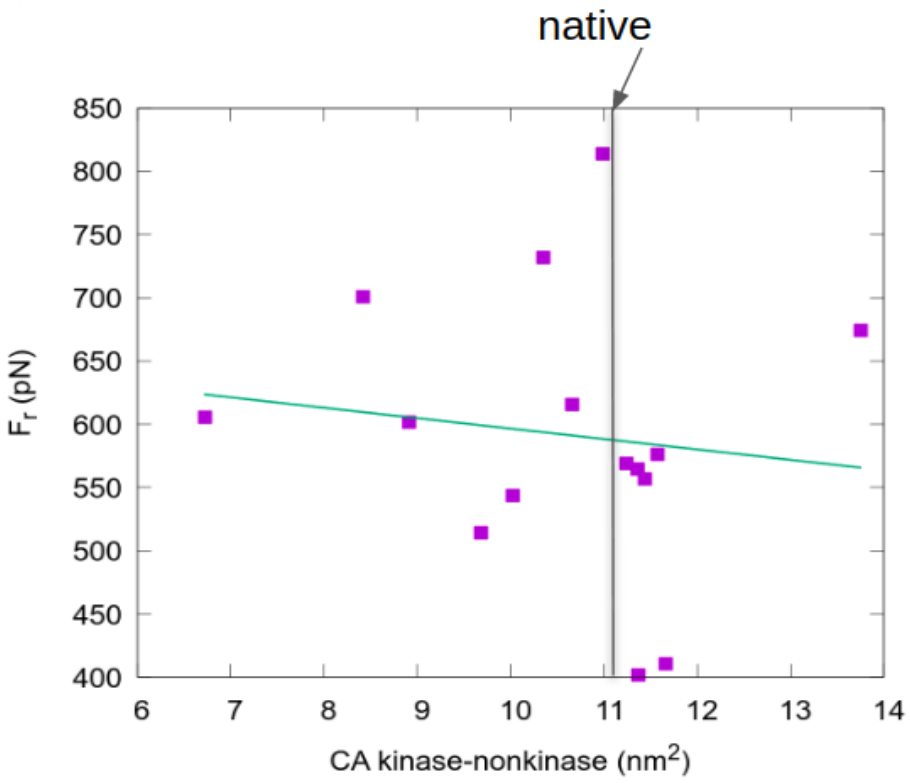

Fig S9: Rupture forces required for enforced activation show no correlation to the contact area of kinase inhibition. All starting frames (native, +7 nm, and +15 nm) are included.