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# Supplementary Information for

## The loops of the N-SH2 binding cleft do not serve as allosteric switch in SHP2 activation

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**This PDF file includes:**

Figs. S1 to S5
Legend for Movie S1
SI References

**Other supplementary materials for this manuscript include the following:**

Movie S1

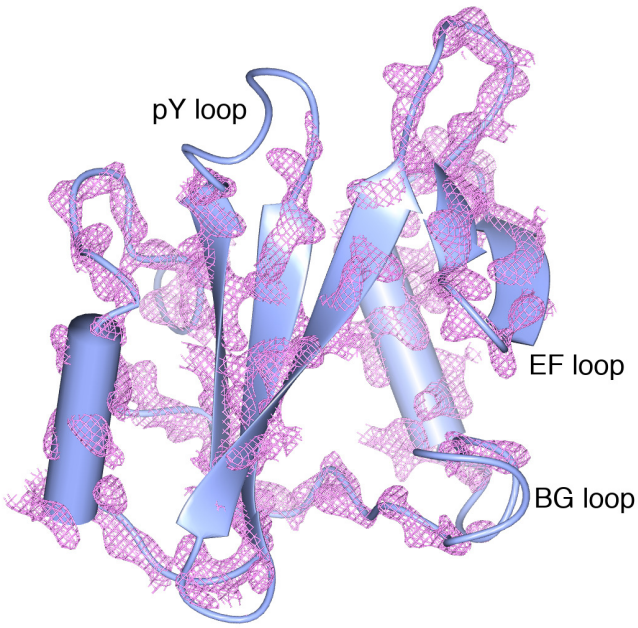


**Fig. S1.** Electron density map (threshold level 0.6 e/Å$^3$) of the N-SH2 domain backbone as obtained from the crystal structure of autoinhibited SHP2 (PDB ID 2SHP) (1). The positions of the flexible loops (pY loop, EF loop, and BG loop) are indicated by the labels.

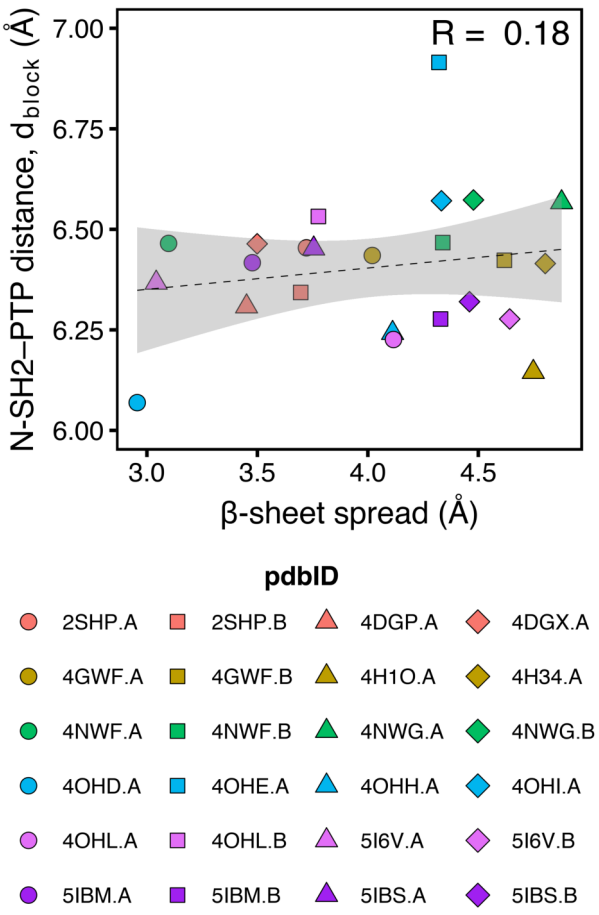


**Fig. S2.** Correlation plot between the N-SH2 central $\beta$-sheet spread and the N-SH2 blocking loop distance from the catalytic PTP loop, $d_{\text{block}}$ (Asp$^{61}$ C$\alpha$–Ala$^{461}$ C$\alpha$ distance), as taken from crystal structure of autoinhibited SHP2, comprising either wild type or functional mutants (1–4).

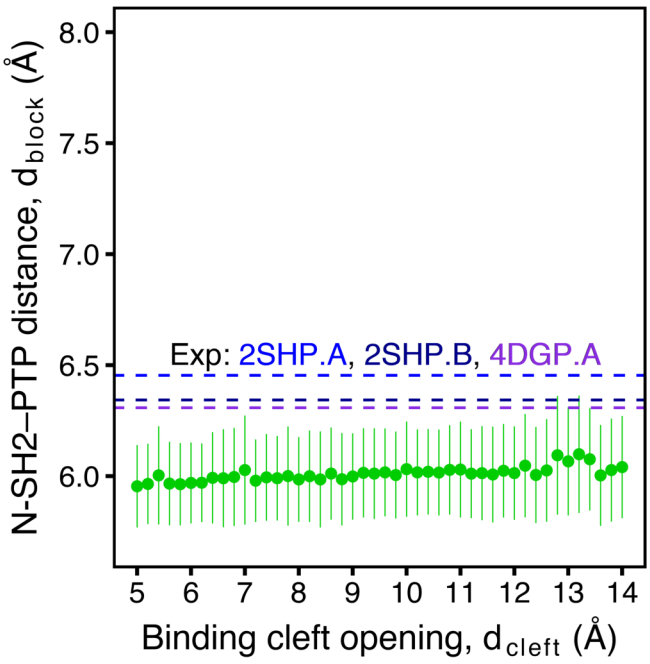


**Fig. S3.** Dot plot of the N-SH2 blocking loop distance from the catalytic PTP loop, $d_{\text{block}}$ (Asp$^{61}$ C$\alpha$–Ala$^{461}$ C$\alpha$ distance), as a function of the N-SH2 binding cleft opening, $d_{\text{cleft}}$ (Gly$^{67}$ C$\alpha$–Lys$^{89}$ C$\alpha$ distance), as obtained from umbrella sampling simulations of autoinhibited SHP2 in water. The bars indicate the RMS fluctuation of $d_{\text{block}}$, that remains below the experimental $d_{\text{block}}$ distances observed in autoinhibited structures of wild-type SHP2 (PDB ID 2SHP and 4DGP) (1, 2).

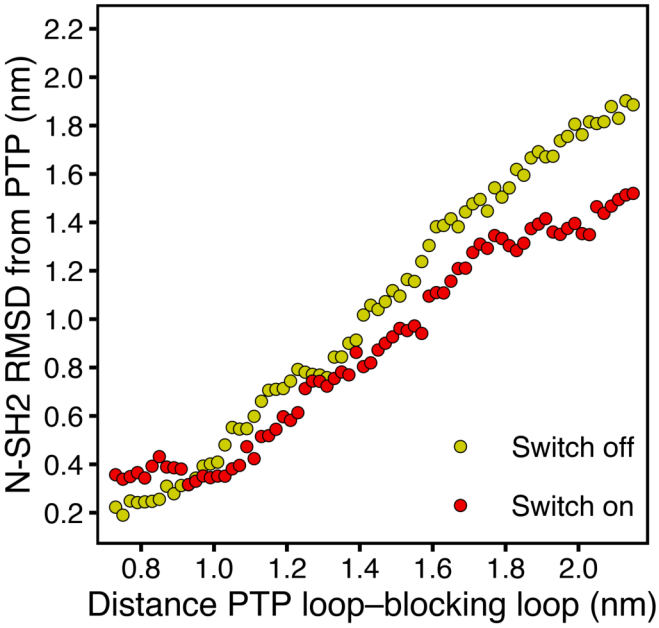


**Fig. S4.** C$\alpha$ root mean-square deviation (C$\alpha$-RMSD) of the N-SH2 domain relative to its position in the autoinhibited structure of SHP2, calculated after the roto-translational least-squares fitting of the PTP domain (C$\alpha$ atoms). The RMSD is plotted as a function of the center-of-mass distance between the blocking loop and the catalytic PTP loop, taken as reaction coordinate in pulling simulation. The N-SH2 binding cleft was restrained either in closed (switch off, yellow dots) or in open (switch on, red dots) conformation.

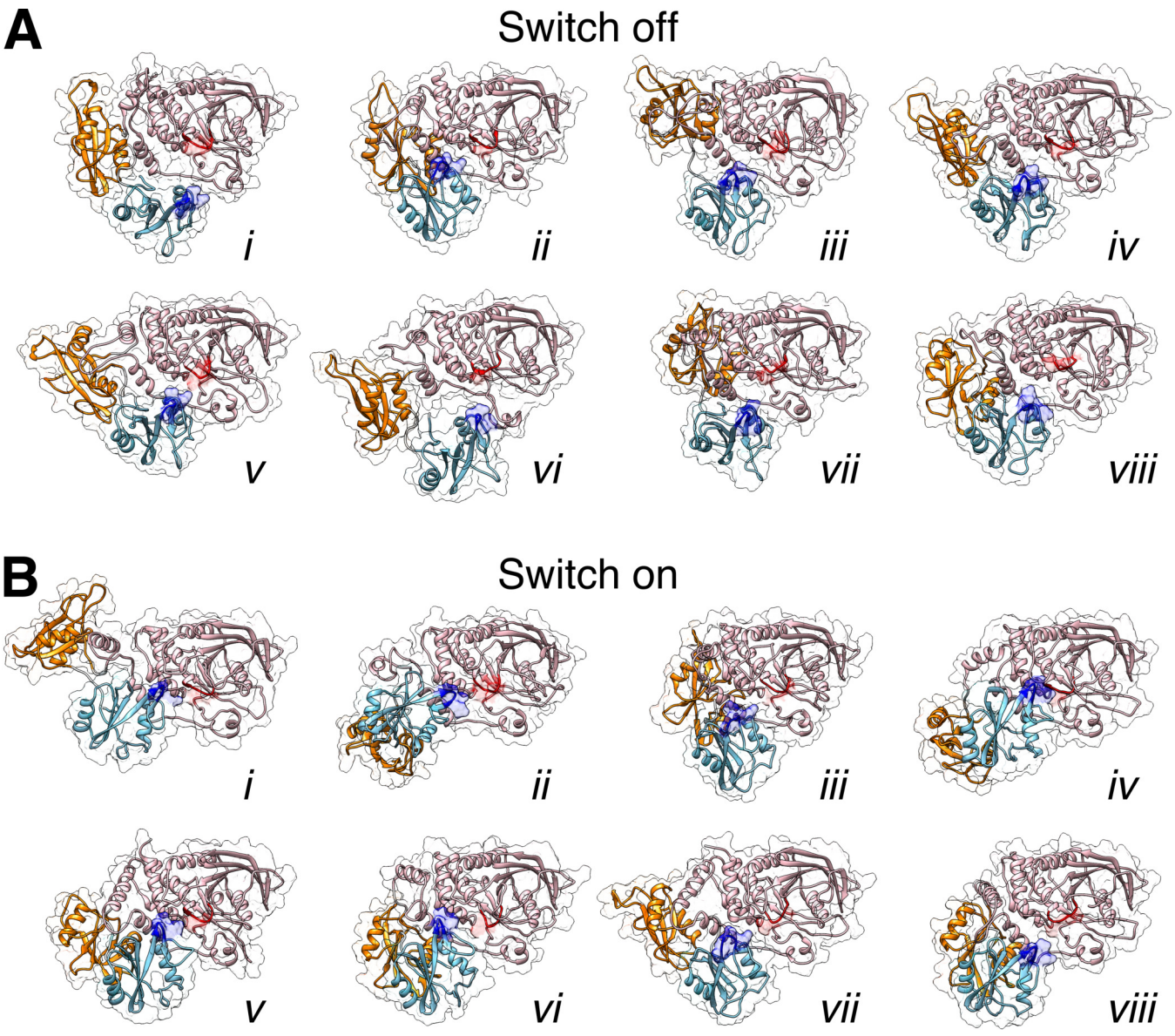


**Fig. S5.** Open and active structures of SHP2 as obtained by 16 independent pulling simulations coupled with simulated tempering, revealing a solvent-exposed active site (red cartoon) on the PTP domain (pink cartoon). Center-of-mass pulling simulations were carried out with restraining the binding cleft of the N-SH2 domain (cyan cartoon) either in closed (A, switch off, *i–viii*) or in open (B, switch on, *i–viii*) conformation. The final position of N-SH2 differs among independent simulation runs, indicative of a large accessible conformational space of activated SHP2. The N-SH2 domain is colored in cyan, the C-SH2 domain in orange, the PTP domain in pink, the PTP catalytic site in red, and the N-SH2 blocking loop in blue.

**Movie S1. Typical trajectory of the opening and activation of SHP2 as obtained by pulling simulation coupled with simulated tempering. The N-SH2 domain (cyan cartoon) moved from its position in the autoinhibited state to a different position relative to the PTP (pink) and the C-SH2 (orange) domain. The blocking loop (blue) leaves the catalytic site (red), making it solvent-accessible. The N-SH2 domain is colored in cyan, the C-SH2 domain in orange, the PTP domain in pink, the PTP catalytic site in red, and the N-SH2 blocking loop in blue.**

## References


1. P Hof, S Pluskey, S Dhe-Paganon, MJ Eck, SE Shoelson, Crystal structure of the tyrosine phosphatase SHP-2. *Cell* **92**, 441–450 (1998).
2. ZH Yu, et al., Structural and mechanistic insights into LEOPARD syndrome-associated SHP2 mutations. *J Biol Chem* **288**, 10472–10482 (2013).
3. JR LaRochelle, et al., Structural and functional consequences of three cancer-associated mutations of the oncogenic phosphatase SHP2. *Biochemistry* **55**, 2269–2277 (2016).
4. ZH Yu, et al., Molecular basis of gain-of-function LEOPARD syndrome-associated SHP2 mutations. *Biochemistry* **53**, 4136–4151 (2014).