Table S1. Summary of simulation systems.

System		NtA	αC-helix	A-loop	
BI		pS446	OUT	Collapsed	
BA		pS446	IN	Extended	-
BI/BI	BI	pS446	OUT	Collapsed	-
	BI	pS446	OUT	Collapsed	-
BI/BA	BI	pS446	OUT	Collapsed	-
	BA	pS446	IN	Extended	pT599 + pS602
BA/BA	BA	pS446	IN	Extended	pT599 + pS602
	BA	pS446	IN	Extended	pT599 + pS602
CI		-	OUT	Collapsed	-
CA		-	IN	Extended	-
BI/CI	BI	pS446	OUT	Collapsed	-
	CI	-	OUT	Collapsed	-
BI/CA	BI	pS446	OUT	Collapsed	-
	CA	-	IN	Extended	pT491 + pS494

B-Raf inactive (BI); B-Raf activate (BA); C-Raf inactive (CI); C-Raf active (CA).

Figure S1. Raf kinase domain conformations. The structures were modeled based on the B-Raf crystal structures (left panel). The inactive Raf kinase domain (KD) has a "collapsed" A-loop and "OUT" αC-helix (upper right panel). The active Raf kinase domain has an "extended" A-loop and "IN" αC-helix (lower right panel). In the inactive conformation, the hydrophobic interactions between the A-loop and the hydrophobic surface in the N-lobe help to confine the "collapsed" A-loop.

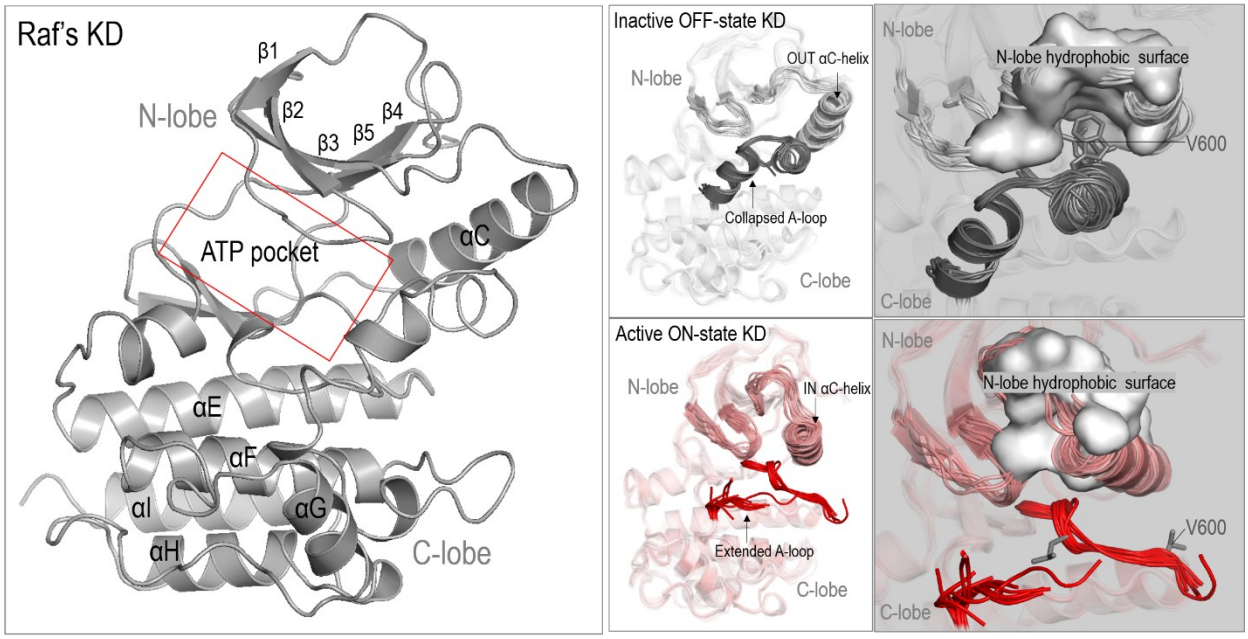

Figure S2. Cα distances between the N-lobe residue K483 (K375 in C-Raf) and the C-lobe residues (residues 550-570 in αE, 634-651 in αF, 686-697 in αH, 706-720 in αI for B-Raf, residues 442-462 in αE, 526-543 in αF, 579-589 in αH, 589-612 in αI for C-Raf). BI refers to B-Raf with inactive conformation, CI refers to C-Raf with inactive conformation, where B and C stand for B-Raf and C-Raf respectively. BA, CA refer to the respective active conformations.

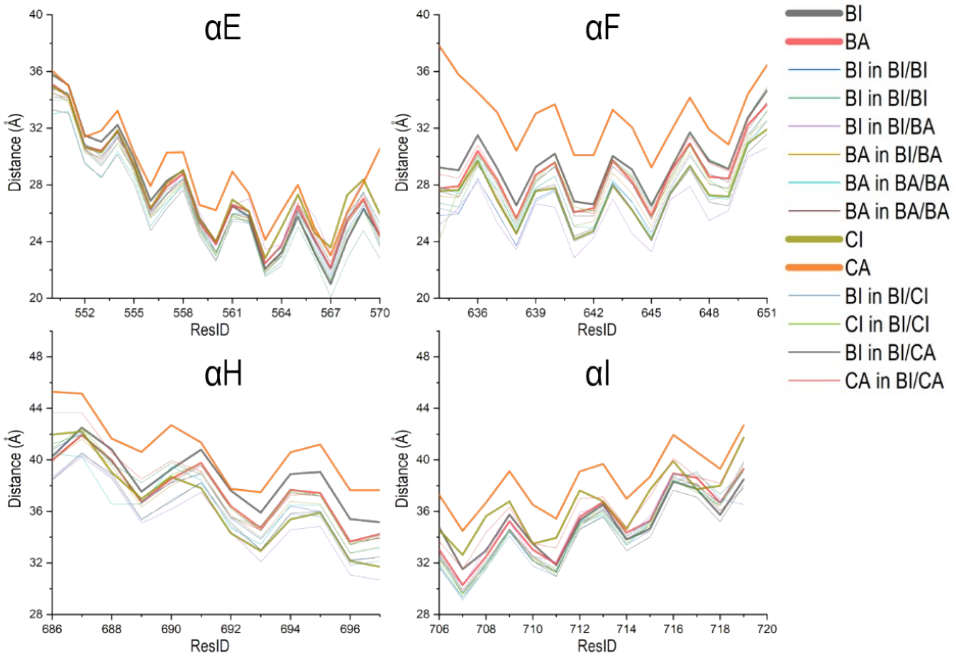

Figure S3. Cα distances for (A) V471-F583 interactions in C-spine and (B) H574-F595 interactions in R-spine. In the violin plots, the white dots denote the median, the thick black lines denote the data ranging from 25% to 75%, and the curve denote the probability density of the data. Staples in *x*-axis denote the dimeric configurations. BI refers to B-Raf with inactive conformation, CI refers to C-Raf with inactive conformation, where B and C stand for B-Raf and C-Raf respectively. BA, CA refer to the respective active conformations.

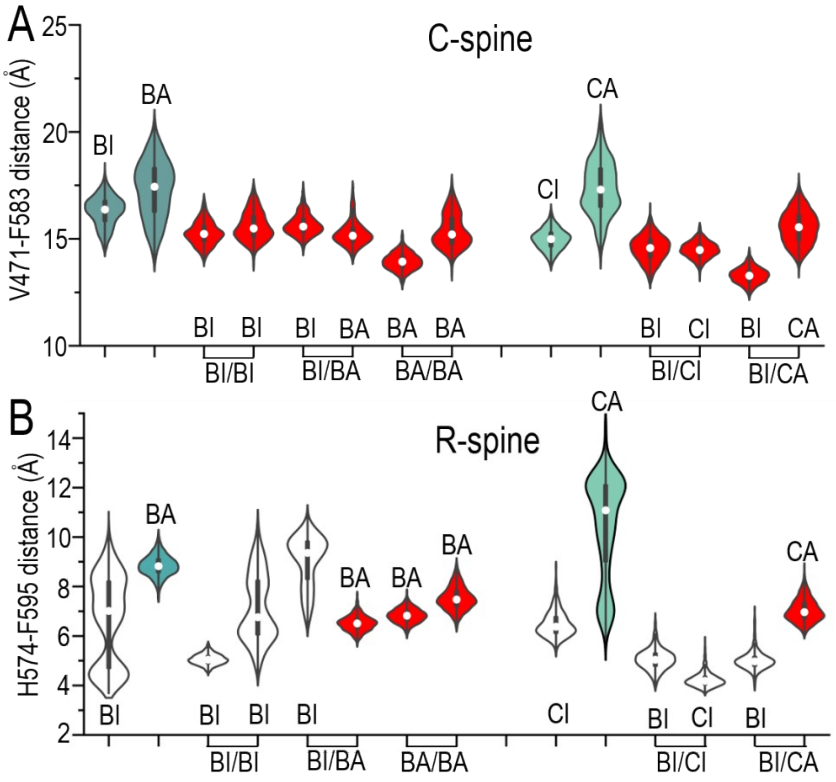

Figure S4. Snapshots showing the disruptions of V600/N-lobe interactions for BI in BI/BA and BI in BI/CI. BI refers to B-Raf with inactive conformation, CI refers to C-Raf with inactive conformation, where B and C stand for B-Raf and C-Raf respectively. BA, CA refer to the respective active conformations.

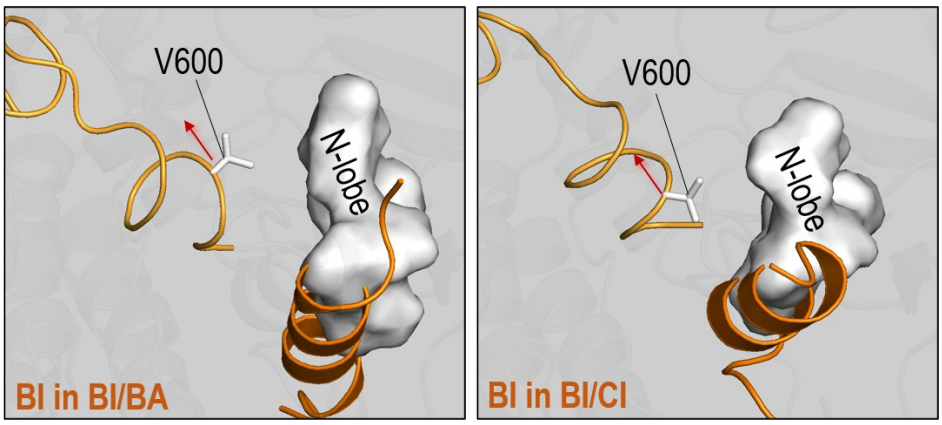

Figure S5. Distances between T599 and S602 in A-loop and D576 in HRD motif. (A) Averaged distances of the residue pairs for T599-D576 and S602-D576 in the dimerized inactive Raf kinases. The distance between S602 (S494 in C-Raf) in the A-loop and D576 (D468 in C-Raf) in the HRD motif is shorter than the distance of T599 (T491 in C-Raf) to D576. Error bars denote the standard deviation. (B) Time-dependent S602-D576 distances in B-Raf monomer (BI) and C-Raf monomer (CI). BI refers to B-Raf with inactive conformation, CI refers to C-Raf with inactive conformation, where B and C stand for B-Raf and C-Raf respectively. BA, CA refer to the respective active conformations.

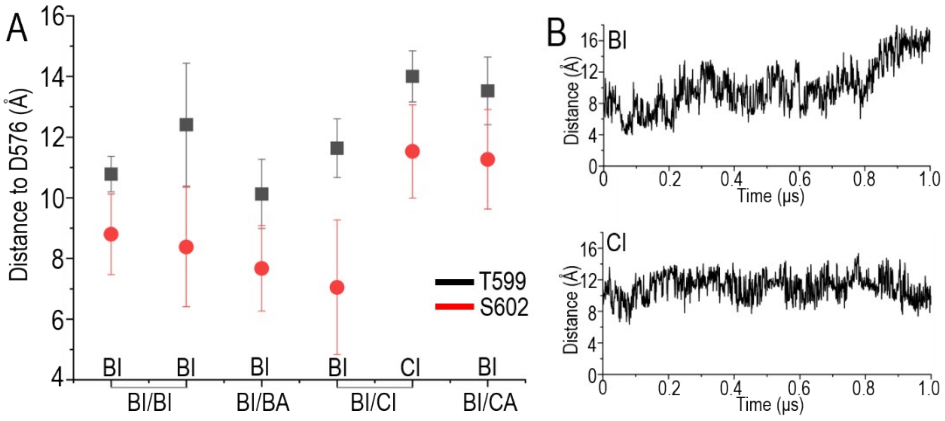

Figure S6. The disruptions of the intramolecular π-π stacking and its replacements by the intermolecular π-π stacking at the dimer interface. BI refers to B-Raf with inactive conformation, CI refers to C-Raf with inactive conformation, where B and C stand for B-Raf and C-Raf respectively. BA, CA refer to the respective active conformations.

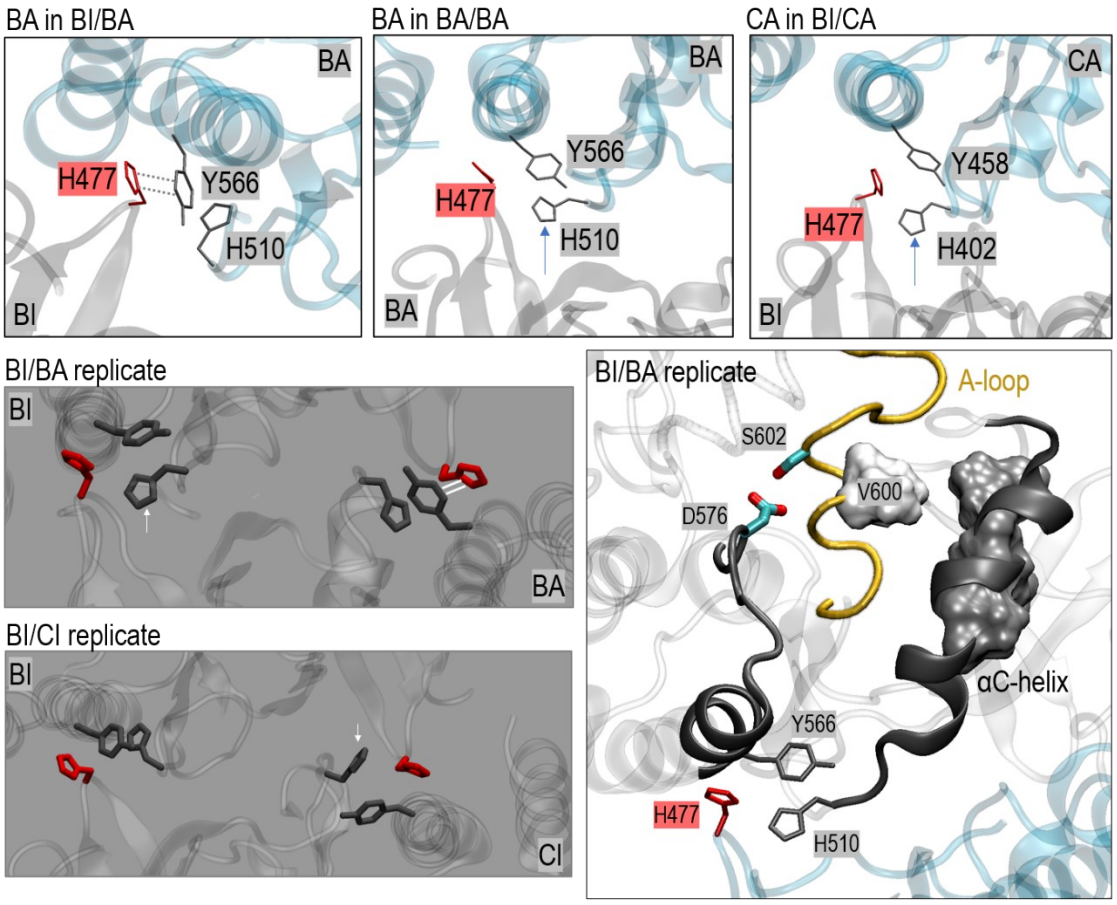

Figure S7. Modeling of Raf dimer with the NtB motif. The NtB-NtA motif orients towards and is sufficiently long to interact with another kinase domain in the dimer.

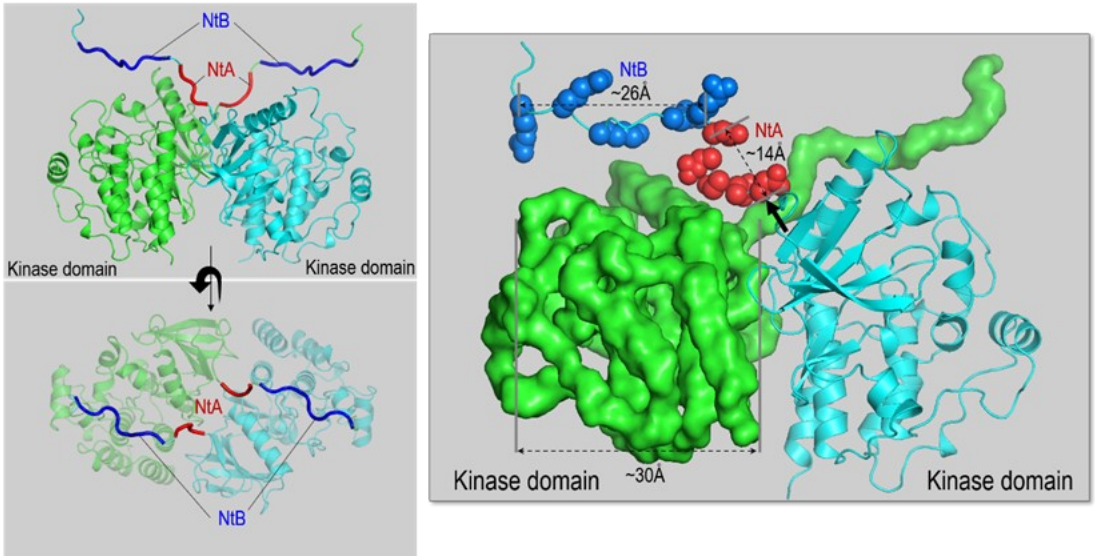

Figure S8. Y566-H510 intermolecular π-π stacking in the Raf crystal and cryo-EM structures.