Supplementary Information

Allosteric Activation of FGFR2 Kinase in Endometrial Cancer: Insights from Gaussian Accelerated Molecular Dynamics and Markov State Model

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Table S1. Network features calculated using the webPSN 2.0 server.

Parameter	wt_unphos	wt_phos	N549K	K659E
Number of Linked Nodes	**260**	**261**	**267**	**262**
Number of Links	**293**	**310**	**326**	**314**
Number of Hubs	**33**	**43**	**65**	**45**
Number of Links mediated by Hubs	**132**	**155**	**215**	**168**
Number of Communities	**9**	**11**	**12**	**11**
Number of Nodes involved in Communities	**63**	**66**	**83**	**70**
Number of Links involved in Communities	**82**	**92**	**121**	**93**

Table S2. The mean RMSD values of the backbone protein and intersegment regions. The standard deviations and error of the mean are also shown. All are calculated using the block average method.

Inter-segment regions	Wt_unphos		Wt_phos		N549K		K659E	
	Mean ± SD	SEM	Mean ± SD	SEM	Mean ± SD	SEM	Mean ± SD	SEM
Backbone	2.97 ± 0.64	0.05	2.69 ± 0.46	0.03	2.37 ± 0.50	0.03	2.11 ± 0.48	0.03
Radius of gyration	19.47 ± 0.12	0.00	19.81 ± 0.11	0.00	19.77 ± 0.09	0.00	19.57 ± 0.14	0.01
SASA	464.49 ±22.18	1.74	481.05 ± 12.58	1.38	467.73 ± 14.49	1.13	473.08 ± 12.76	1.00
A-loop	1.51 ± 0.79	0.06	2.05 ± 0.30	0.03	1.42 ± 0.13	0.01	1.31 ± 0.23	0.01
DFG	0.26 ± 0.03	0.00	0.25 ± 0.06	0.00	0.24 ± 0.04	0.00	0.33 ± 0.07	0.00
C-loop	0.40 ± 0.04	0.00	0.44 ± 0.02	0.00	0.41 ± 0.03	0.00	0.35 ± 0.03	0.00
HRD motif	0.15 ± 0.00	0.00	0.16 ± 0.01	0.00	0.13 ± 0.00	0.00	0.16 ± 0.01	0.00
Hinge	0.43 ± 0.03	0.00	0.34 ± 0.01	0.00	0.36 ± 0.01	0.00	0.47 ± 0.09	0.00
Molecular brake	0.90 ± 0.19	0.01	0.27 ± 0.01	0.00	0.33 ± 0.02	0.00	0.33 ± 0.07	0.00
P-loop	0.84 ±	0.01	0.94 ±	0.04	1.75 ±	0.01	1.30 ±	0.01

	0.25		0.56		0.22		0.24	
R-spine	0.56 ± 0.09	0.00	0.60 ± 0.04	0.00	0.48 ± 0.08	0.00	0.41 ± 0.05	0.00
C-spine	1.45 ± 0.27	0.02	1.06 ± 0.14	0.01	0.77 ± 0.12	0.00	1.16 ± 0.24	0.01
Alpha DE loop	1.96 ± 1.26	0.09	3.10 ± 0.47	0.03	2.73 ± 0.94	0.07	2.17 ± 1.01	0.07

Table S3. The H-bond occupancy among Glu565, Asn549, and Lys641 residues of the molecular brake region.

Binding couples		**Molecular dynamics**	
Acceptor	**Donor**	**Distance (Å)**	**Occupancy (%)**
Wt_unphos			
Glu@OE2	Asn@ND2	2.81	39.21
Glu@OE1	Asn@ND2	2.81	32.12
Glu@OE2	Asn@N	2.86	27.61
Glu@OE1	Asn@NH1	2.86	23.44
Asn@O	Glu@NH2	2.89	31.15
GLU@OE2	LYS@HZ1	2.78	15.6
GLU@OE2	LYS@HZ3	2.78	15.5
GLU@OE2	LYS@HZ2	2.78	15.4
GLU@OE1	LYS@HZ3	2.78	13.5
GLU@OE1	LYS@HZ1	2.78	13.4
GLU@OE1	LYS@HZ2	2.78	13.3
Wt_phos			
GLU@OE1	ASN@ND2	2.80	44.05
GLU@OE2	ASN@ND2	2.80	43.78
GLU@OE1	ASN@N	2.86	28.20
GLU@OE2	ASN@N	2.86	28.11
ASN@O	GLU@N	2.89	30.09
GLU@OE1	LYS@NZ	2.78	15.14
N549K			
GLU@OE2	LYS@N	2.84	26.65
GLU@OE1	LYS@N	2.84	22.84
K659E			
GLU@OE1	ASN@ND2	2.80	21.34

GLU@OE2	ASN@ND2	2.80	20.45
ASN@O	GLU@N	2.89	23.83

Table S4. The state population obtained for each state from the Markov state model. Each state was mentioned as mentioned as active like or inactive like conformation. Active like – Open A-loop, open P-loop, -*in* αC helix. Inactive like – closed A-loop, restricted P-loop and -*out* αC helix.

Metastable states	Probability of Occurrence
Wt_unphos	
State 1 (Inactive like)	0.3352
State 2 (Inactive like)	0.3254
State 3 (Inactive like)	0.3394
Wt_phos	
State 1 (partially active)	0.1653
State 2 (active like)	0.5120
State 3 (partially active)	0.3226
N549K	
State 1 (partially active)	0.1087
State 2 (active like)	0.5533
State 3 (active like)	0.2281
State 4 (active like)	0.1099
K659E	
State 1 (active like)	0.0787
State 2 (active like)	0.3459
State 3 (active like)	0.5754

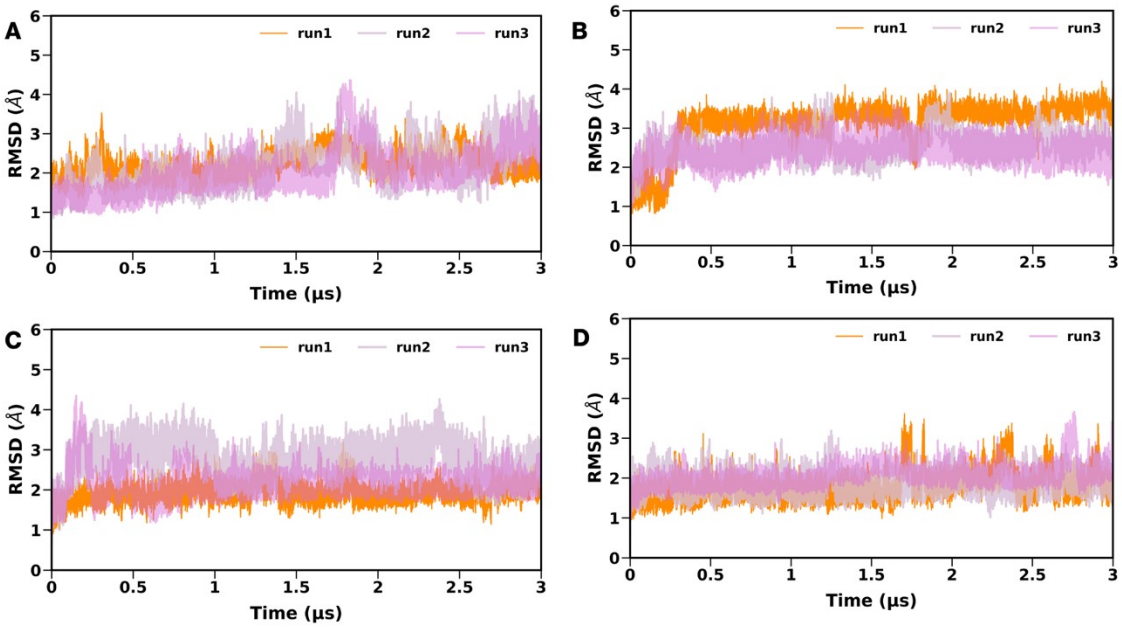

Figure S1. Time evolution root mean square deviation (RMSD) plot of backbone Cα atoms for all the systems. (A) wt_unphos (B) wt_phos (C) N549K (D) K659E.

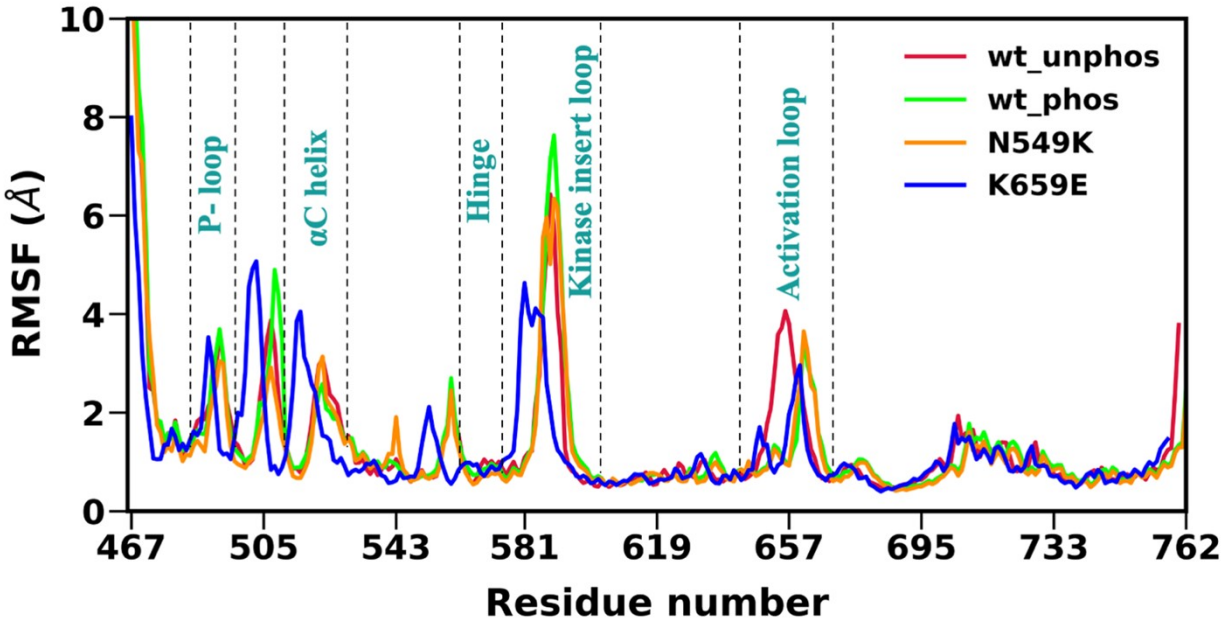

Figure S2. Root mean square fluctuations (RMSF) of Cα atoms for all the systems.

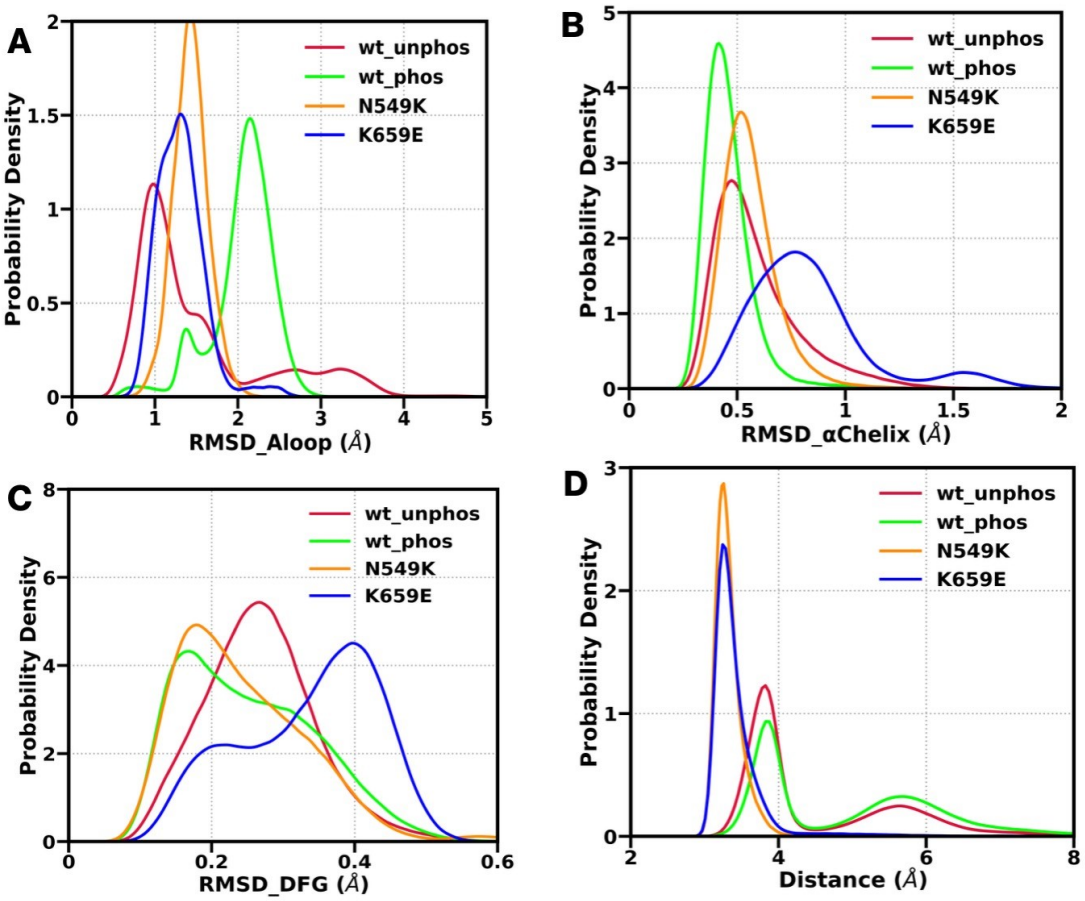

Figure S3. The RMSD Probability density distribution of (A) A-loop (B) αChelix (C) DFG motif (D) Distance between K517 and E534 for all the systems.

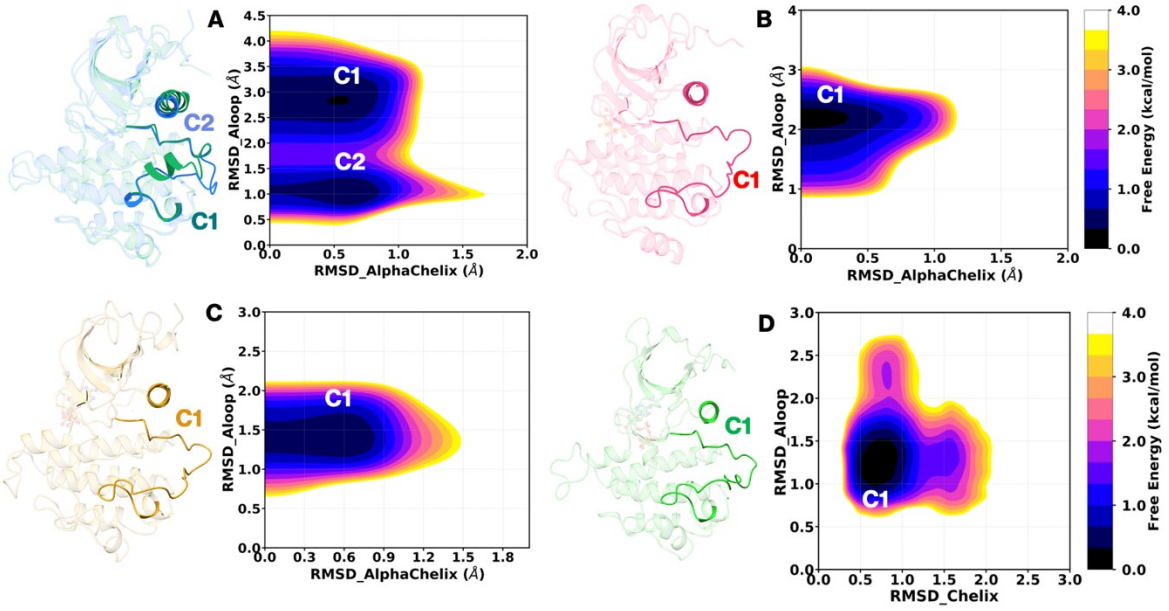

Figure S4. Free energy landscape obtained using two reaction coordinates, RMSD_Aloop and RMSD_αChelix for (A) wt_unphos (B) wt_phos (C) N549K (D) K659E. The corresponding conformations obtained from the low-energy space are shown, respectively.

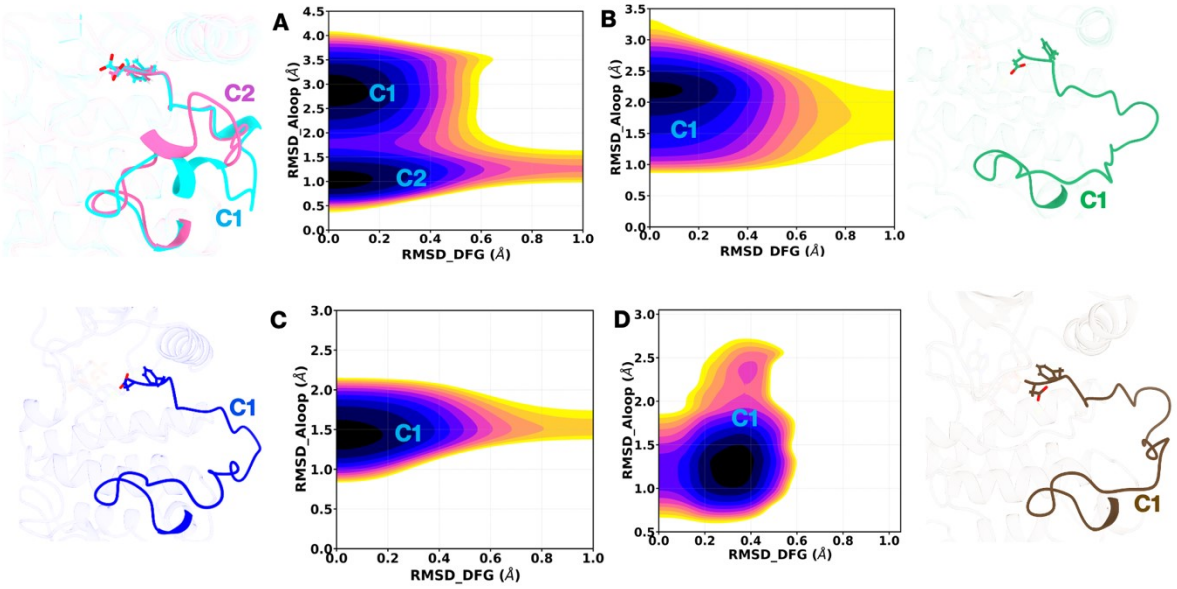

Figure S5. Free energy landscape obtained using two reaction coordinates, RMSD_Aloop and RMSD_DFG motif for (A) wt_unphos (B) wt_phos (C) N549K (D) K659E. The corresponding conformations obtained from the low energy space are shown respectively.

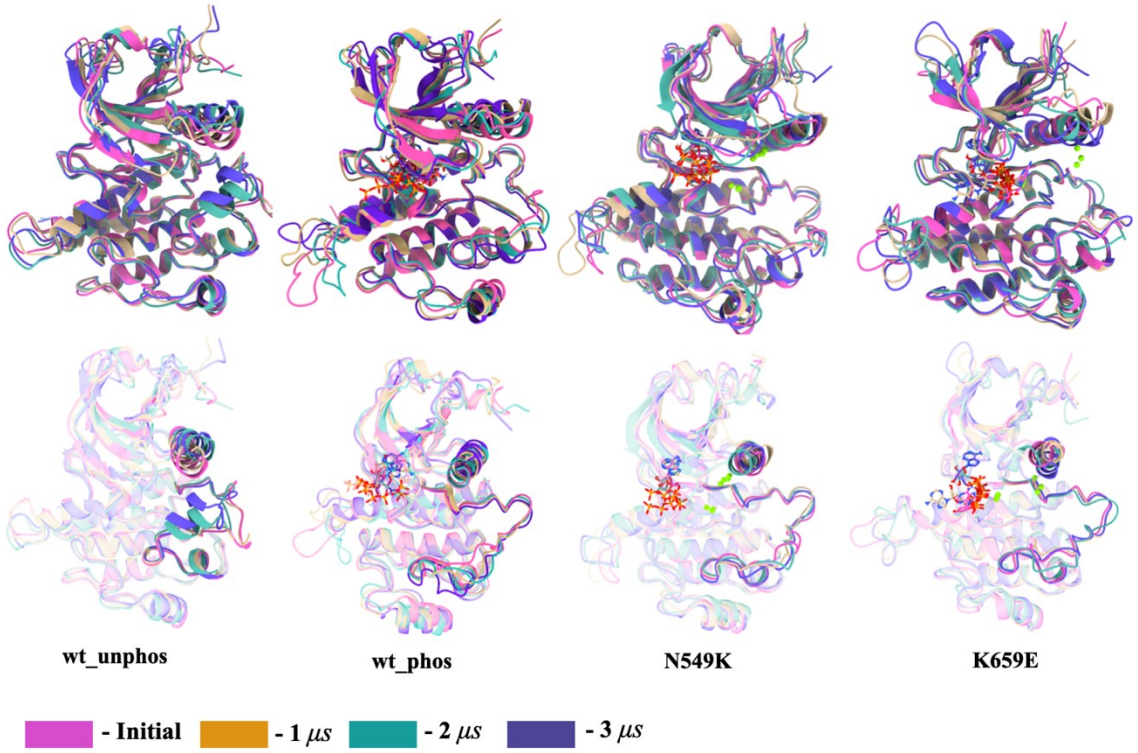

Figure S6. Superimposed structures from different time points of the simulation. The αChelix and A-loop regions are shown separately keeping other regions transparent for a better view.

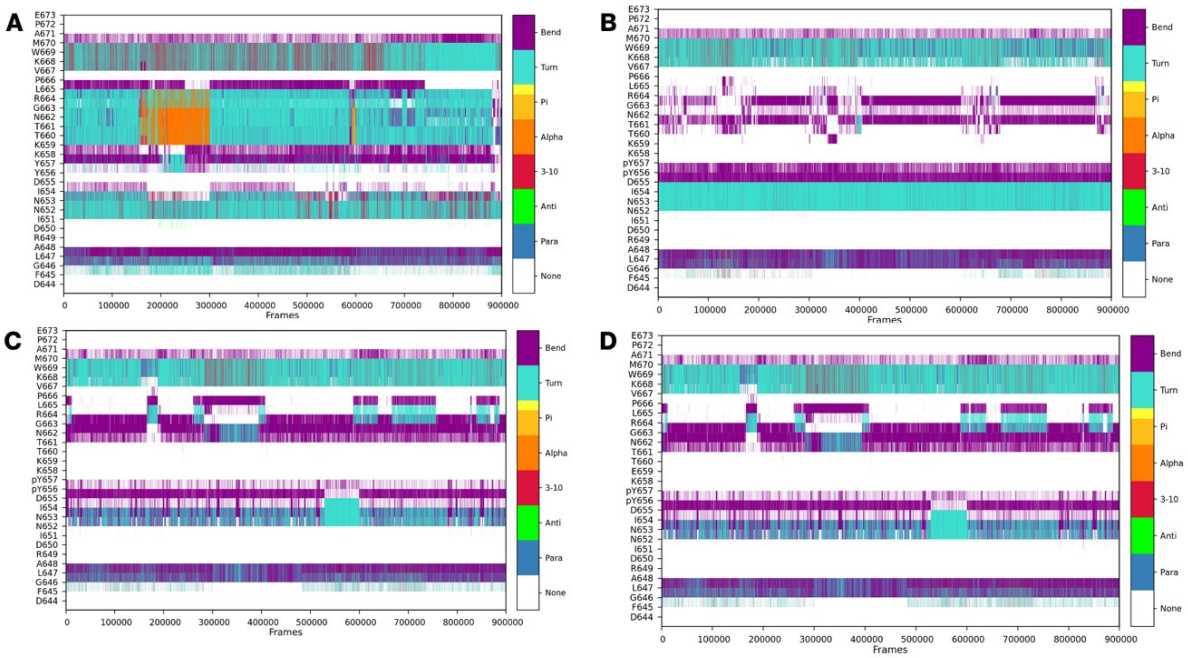

Figure S7. DSSP of activation loop region for (A) wt_unphos (B) wt_phos (C) N549K (D) K659E

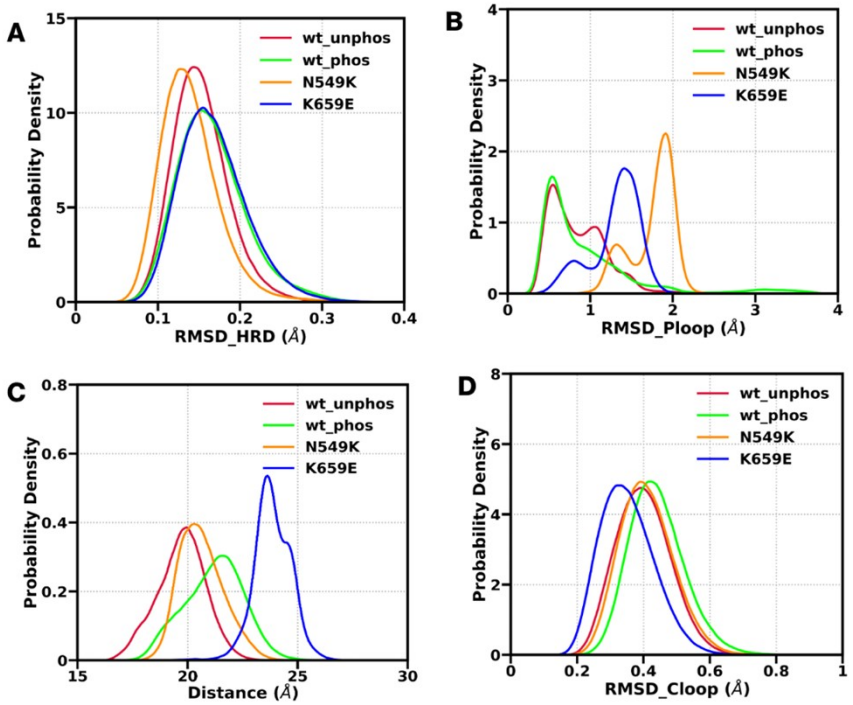

Figure S8. The RMSD Probability density distribution of (A) HRD motif (B) P-loop (C) Distance between HRD and P-loop (D) C-loop.

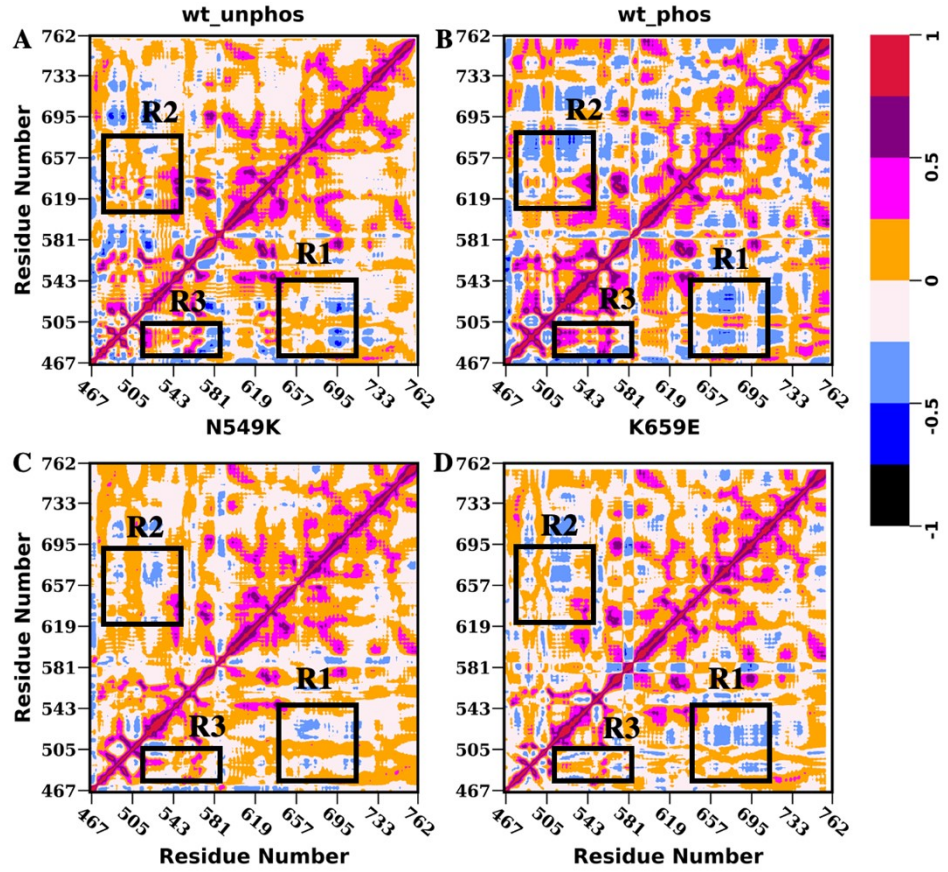

Figure S9. Dynamic cross correlation matrix for (A) wt_unphos (B) wt_phos (C) N549K (D) K659E. Specific regions are highlighted as R1, R2, R3.

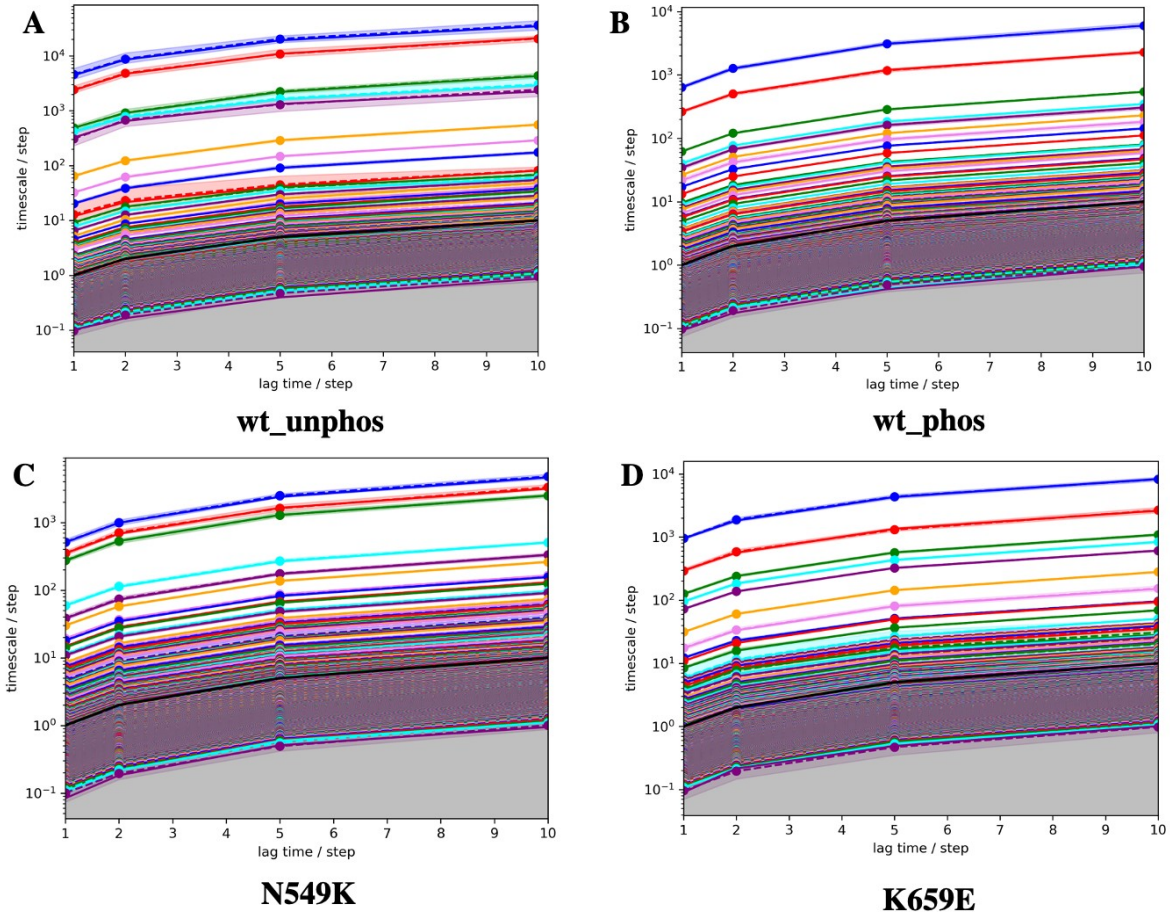

Figure S10. Implied timescale analysis shown for (A) wt_unphos (B) wt_phos (C) N549K (D) K659E

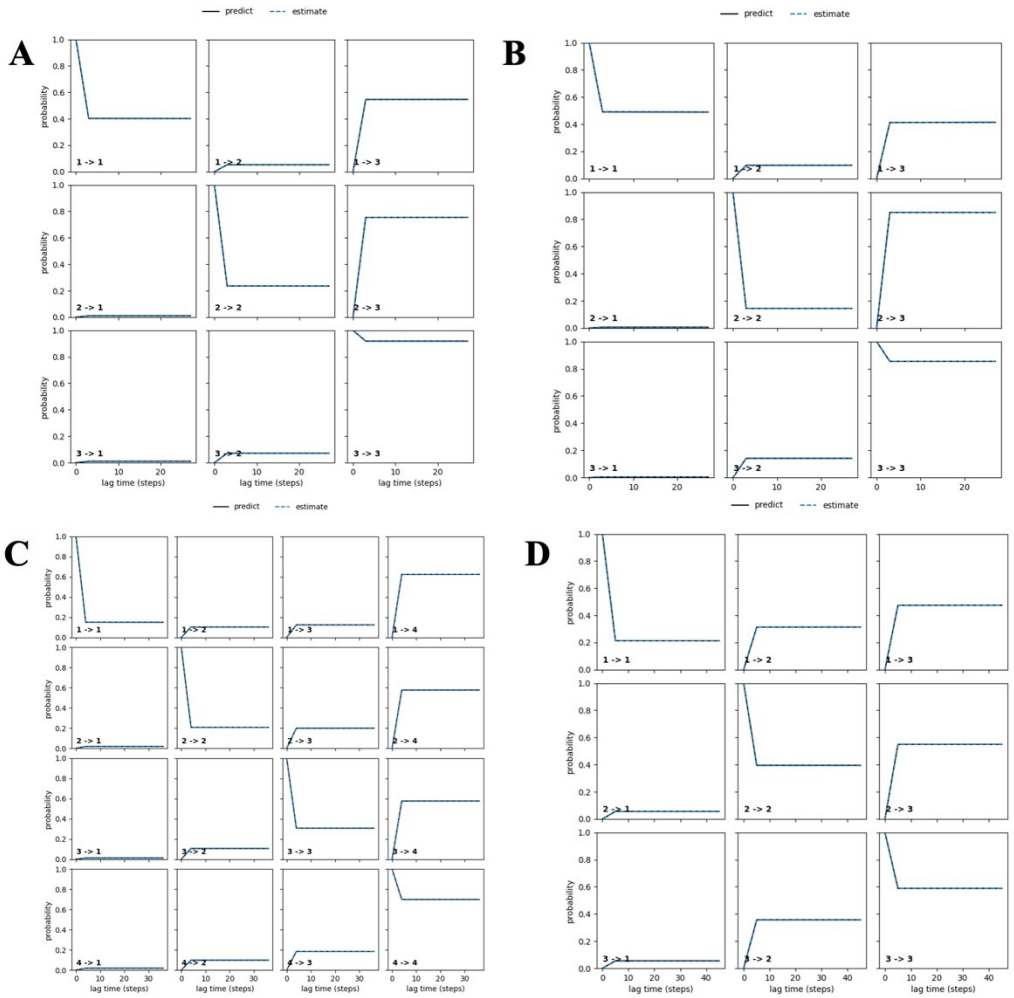

Figure S11. The CK test for (A) wt_unphos, (B) wt_phos, (C) N549K, and (D) K659E systems.

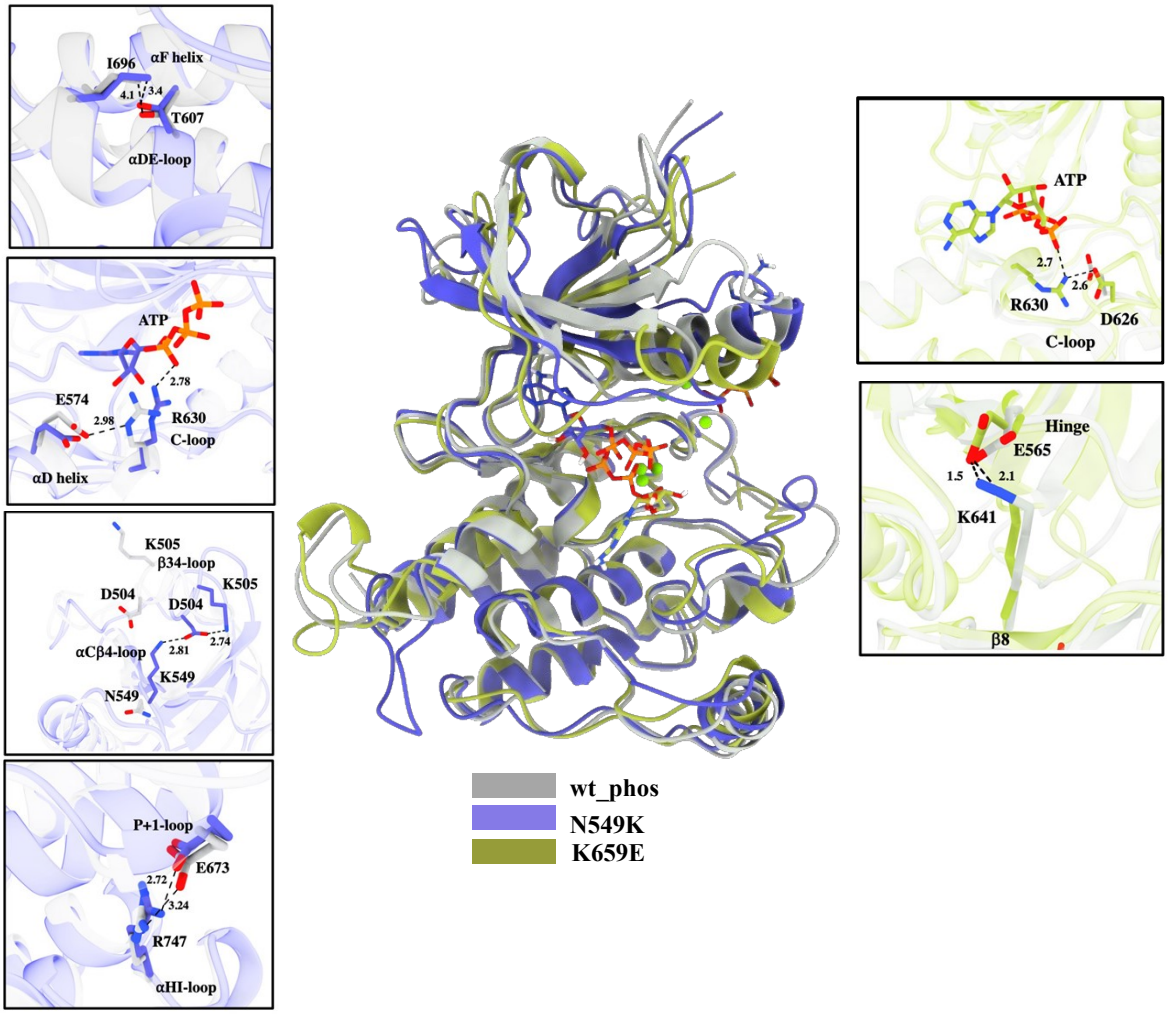

Figure S12. Allosteric interaction regulated by mutation in FGFR2 Kinase. The dominant state conformations from MSM analysis are superimposed for wt_phos (grey color), N549K (violet color), K659E (green color) systems. The left side panel (A-D) shows close up superimposed view of wt_phos and N549K systems with intra domain interactions. The right side panel (E-H) shows close up view of superimposed wt_phos and K659E systems with specific interactions among multiple regions.

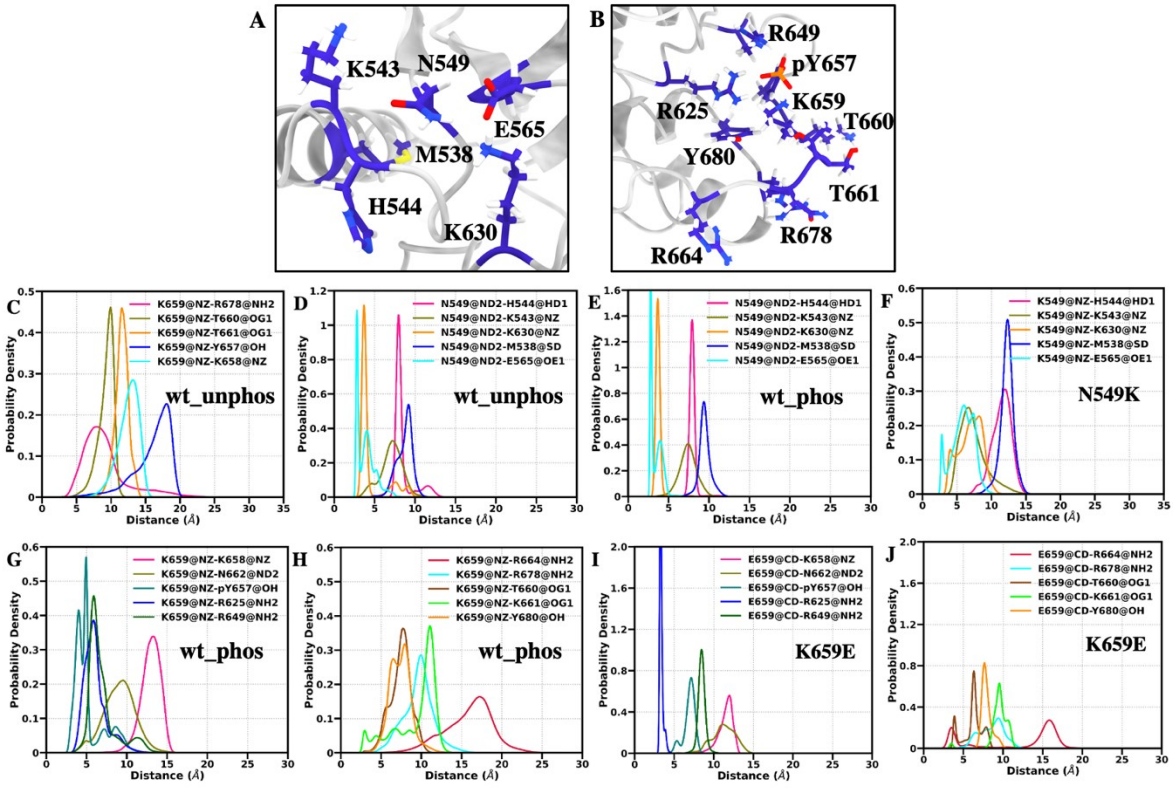

Figure S13. Electrostatic interactions of N/K 549 and K/E 659 residues with nearby residues within the 5 Å range are displayed. A and B show the residues taken into consideration. C-J shows the probability density distribution of distance among residues.

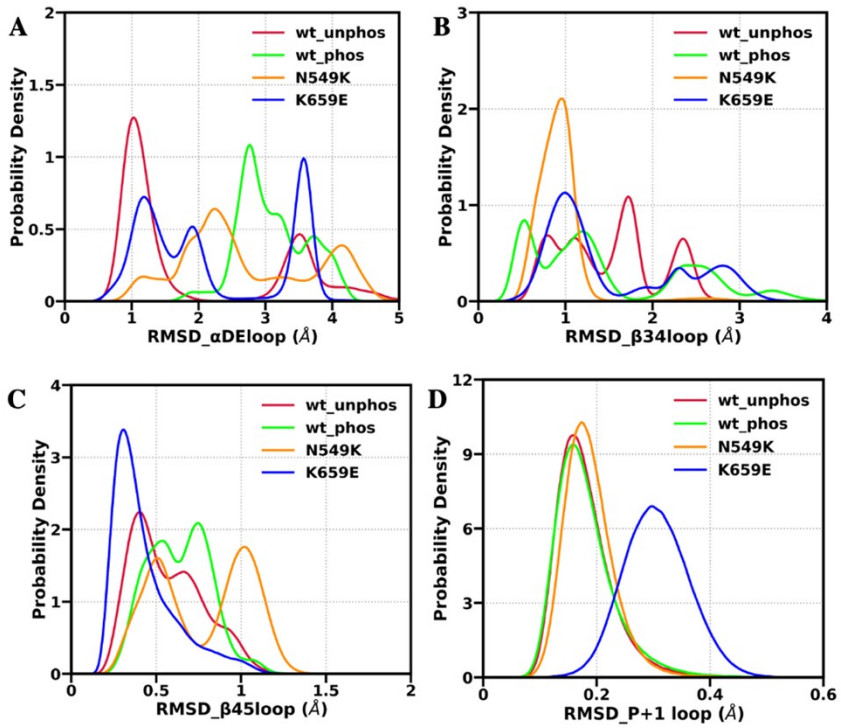

Figure S14. The RMSD Probability density distribution of (A) αDE loop (B) β34 loop (C) β45 loop (D) P+1 loop for all the systems.