

Supporting Information

The importance of domain closure for the auto-activation of ERK2

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Table T1. Solvent accessible surface area of Y185.

System	MD SASA ($\text{\AA}^2$)	X-Ray SASA ($\text{\AA}^2$)
Inactive ^a	6.0 ± 2.3	4.7
Q103A ^a	10.8 ± 4.3	
I84A ^a	10.0 ± 3.8	
L73P ^a	7.3 ± 3.3	
Mono-phosphorylated ^b	71.1 ± 16.2	67.0
Active ^a	71.4 ± 7.8	71.3

a) ERK2

b) ERK1

Table T2. Mean Correlation Coefficients.

System	Lip-Protein	Whole Protein
Inactive ^a	-0.03070	0.19057
Q103A ^a	-0.01390	0.14649
I84A ^a	-0.01068	0.17141
L73P ^a	-0.03262	0.18115
G83A ^a	-0.01299	0.15649
K162M ^a	0.00970	0.15336
Mono-phosphorylated ^b	0.01246	0.17027
Active ^a	0.00524	0.16801

a) ERK2

b) ERK1

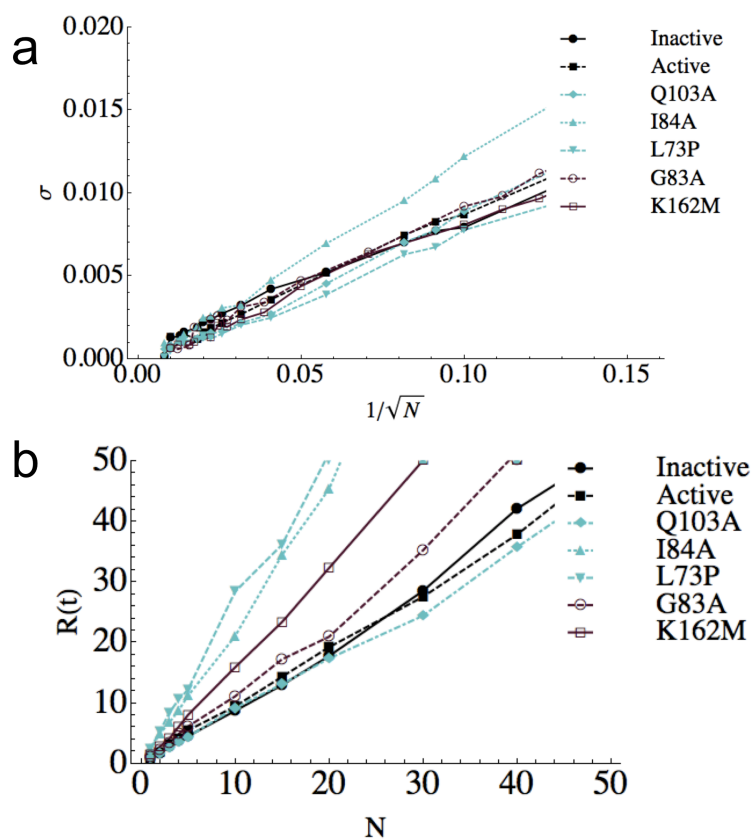


Figure S1. Block based convergence tests of a) the RMSD and b) the variance-covariance matrices (see text).

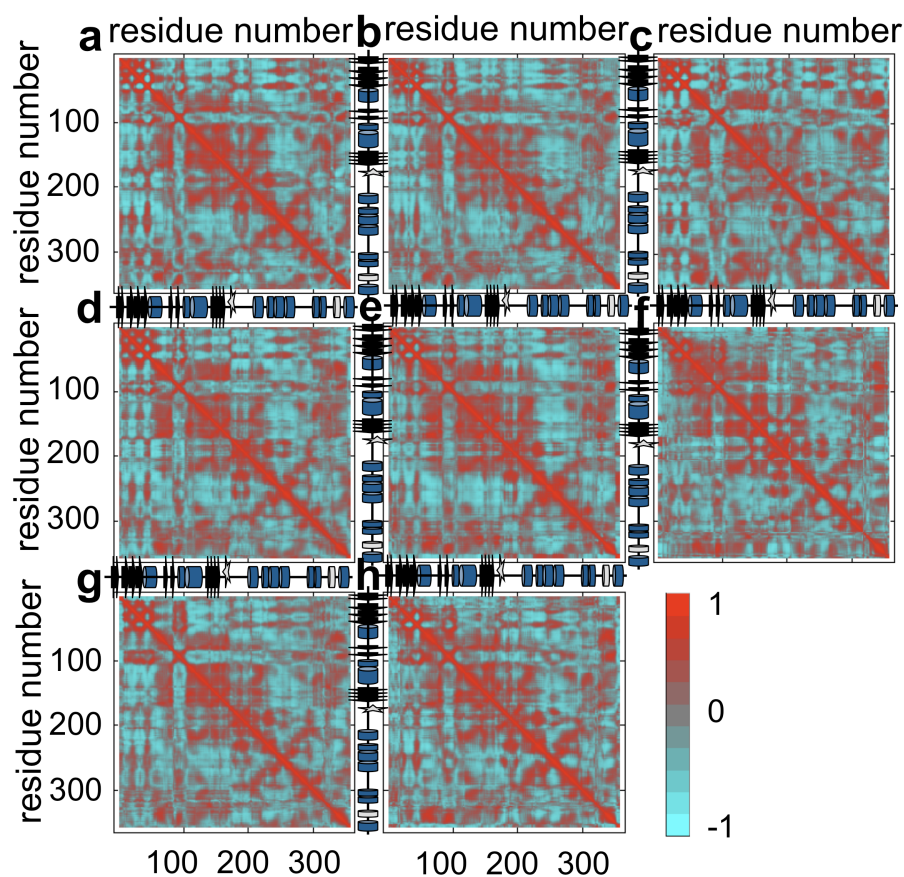


Figure S2. Variance-covariance matrices for: a) inactive ERK2, b) active ERK2, c) monophosphorylated ERK1, d) ERK2 mutant Q103A, e) ERK2 mutant I84A, f) ERK2 mutant L73P g) ERK2 mutant G83A, and h) ERK2 mutant K162M. Secondary structure labels are provided along the axes. The phosphorylation site (TEY motif) is shown by a light grey star, and the 3_{10} helical region of L16 in the active state is shown in light grey.

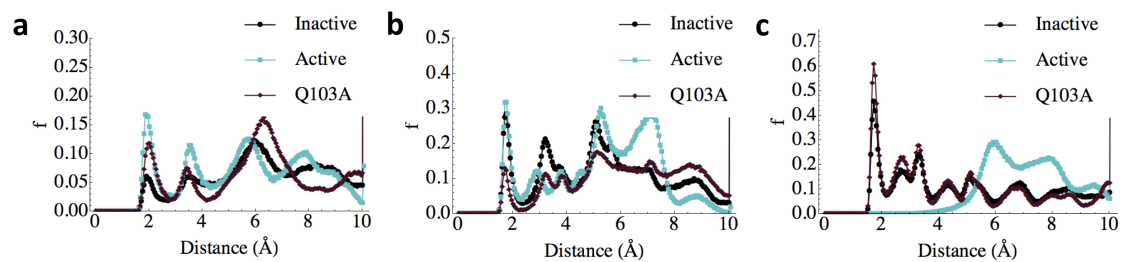


Figure S3. Trajectory-averaged distance histograms for contacts between D334 and a) Q64, b) R68, and c) R170 in the inactive, active and Q103A mutant states.

ERK2	1ERK_A	SAYDNLNKVRVAIKKIS--PFEHQTYCQRTLREIKILLRFRHENIIGINDIIR-APTIEQ	95
ERK1	2ZOQ_A	SAYDHVRKTRVAIKKIS--PFEHQTYCQRTLREIQILLRFRHENVIGIRDILR-ASTLEA	117
P38a	3KF7_A	AAFDTKTGLRVAVKKLSR-PFQSIHAKRTYRELRLKKHMKHENVIGLLDVFTPARSLEE	98
P38d	3COI_A	SAIDKRSGEKVAIKKLSR-PFQSEIFAKRAYRELLLLKKHMQHENVIGLLDVFTPASSLRN	100
JNK3	2OK1_A	AAYDAVLDRNVAIKKLSR-PFQNTQTHAKRAYRELVLKCVNHKNIIISLLNVFTPOKTLEE	101
MEK1	1S9J_A	KVSHKPSGLVMARKLIHL-EIKPAIRN-QIIRELQVLHECNSPYIVGFYGAFYSDGEIS-	80
MEK2	1S9I_A	KVQHRPSGLIMARKLIHL-EIKPAIRN-QIIRELQVLHECNSPYIVGFYGAFYSDGEIS-	90
FGFR1	1EVT_C	-----	
FGFR2	1EV2_E	-----	
FGFR3c	1RY7_B	PPPGGGPMGPTVWVKDGT-GLVPSERVLVGPQRLQVLNASHEDSGAYSCRQRLTQRVLCH	89
ASK1	2CLQ_A	AGRDLNQNVRVIAIKEIP---ERDSRYSQPLHEEIALHKHLKHKNIVQYLGFSSENGFIK-	95
BRAF	1UWJ_A	KG---KWHGDVAVKMLNV-TAPTQQQLQAFKNEVGVLKRTRHVNILFMGYSTKPQLAIV	81
cAPK	1BX6_A	LVKHKESGNHYAMKILDQKQVVKLKQIEHTLNEKRILQAVNFPFLVKLEFSFKDNSNLY-	117
ERK2	1ERK_A	M--KDVYIVQDLMETDLYKLLKT-Q---HLSNDHICYFLYQILRGLKYIHSAN-VLHRDL	148
ERK1	2ZOQ_A	M--RDVYIVQDLMETDLYKLLKS-Q---QLSNDHICYFLYQILRGLKYIHSAN-VLHRDL	170
P38a	3KF7_A	F--NDVYLVTHLMGADLNNIVKC-Q---KLTDHVVQFLIYQILRGLKYIHSAD-IIHRDL	151
P38d	3COI_A	F--YDFYLVMPFMQTDLQKIMG--L---KFSEEKIQYLVYQMLKGLKYIHSAG-VVHRDL	152
JNK3	2OK1_A	F--QDVYLVMEMLDANLCQVIQ--M---ELDHERMSYLLYQMLCGIKHLHSAG-IIHRDL	153
MEK1	1S9J_A	-----ICMEHMDGGSGLDQVLKKAG---RIPEQILGKVSIAVIKGLTYLREKHKIMHRDV	131
MEK2	1S9I_A	-----ICMEHMDGGSGLDQVLKEAK---RIPEEILGKVSIAVLRGLAYLREKHKIMHRDV	141
FGFR1	1EVT_C	-----TDNTKPN---RMPVAPYWTSPKMEKKLHAVPAAKTVKFKCP	39
FGFR2	1EV2_E	-----NSNNKR-----APYWTNTEKMEKRLHAVPAANTVKFRCP	34
FGFR3c	1RY7_B	FSVRVTDAPSSGDDDEGEDEAEDTG---VDTGAPYWTRPERMDKKLLAVPAANTVRFRCP	146
ASK1	2CLQ_A	-----IFMEQVPGGSLALLRSKWGPKDNEQTIGFYTKQILEGLKYLHDNQ-IVHRDI	148
BRAF	1UWJ_A	T-----QWCEGSSLYHHLHIIET--KFEMIKLIDIARQTAQGM DYLHAKS-IIHRDL	130
cAPK	1BX6_A	-----MVMEYVAGGEMFSLRRIG---RFSEPHARFYAAQIVLTTFEYLHSLD-LIYRDL	167
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ERK2	1ERK_A	KPSNLLLNTTC-DLKICDFGLARVADPDHDHTGFLTEYVATRWYRAPEIMLNS--KGYTK	205
ERK1	2ZOQ_A	KPSNLLINTTC-DLKICDFGLARIADPEHDHTGFLTEYVATRWYRAPEIMLNS--KGYTK	227
P38a	3KF7_A	KPSNLAVNEDC-ELKILDFGLARHTDD-----EMTGYVATRWYRAPEIMLNW--MHYNQ	202
P38d	3COI_A	KPGNLAVNEDC-ELKILDFGLARHADA-----EMTGYVVTRWYRAPEVILSW--MHYNQ	203
JNK3	2OK1_A	KPSNIVVKSDC-TLKILDFGLARTAGTSF---MMPYVYVTRYRAPEVILG---MGYKE	205
MEK1	1S9J_A	KPSNILVNSRG-EIKLCDFGVSGQLIDS-----MANSFVGTRSYMSPERLQG---THYSV	182
MEK2	1S9I_A	KPSNILVNSRG-EIKLCDFGVSGQLIDS-----MANSFVGTRSYMAPERLQG---THYSV	192
FGFR1	1EVT_C	SSGTPNPTLRW-LKNGKEFKPDHRIGG-----YKVRYATWSIIMDSVVPD--KGNYS	89
FGFR2	1EV2_E	AGGNPMPMTMRW-LKNGKEFKQEHRIIG-----YKVRNQHWSLIMESVVPD--KGNYS	84
FGFR3c	1RY7_B	AAGNPTPSISW-LKNGREFRGEHRIG-----IKLRHQQWSLVMESVVPD--RGNYS	196
ASK1	2CLQ_A	KGDNVLINTYSGVLKISDFGTSKRLAGIN---PCTETFTGTLYMAPEIIDKGP-RGYGK	204
BRAF	1UWJ_A	KSNNIFLHEDL-TVKIGDFGLATEKSRWSG-SHQFEQLSGSILWMAPEVIRMQDKNPYSF	188
cAPK	1BX6_A	KPENLLIDQQG-YIQVTDGFGAKRVKG-----RTWTLCGTPEYLAPEIILSK---GYNK	217
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Figure S4. Sequence alignment of MAKs. The protein name and PDB access code are shown on the left. The sequence for cAPK is included at the bottom for comparison with Ref. (54). The residues mutated in this study are highlighted in yellow.

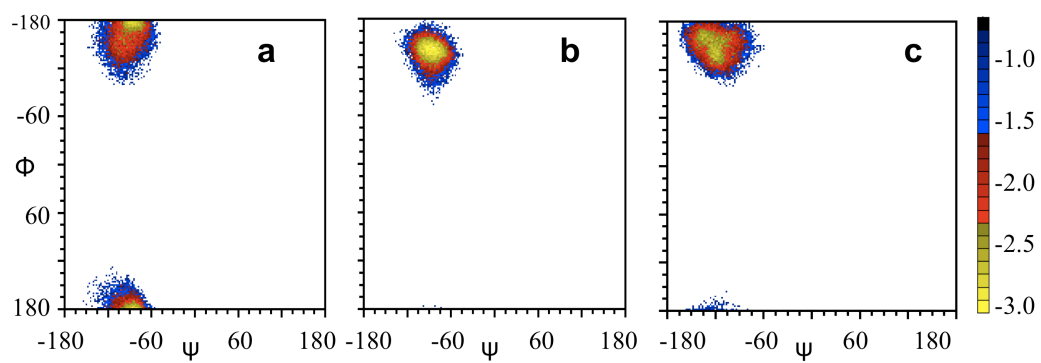


Figure S5. Free energy surfaces (in kcal/mol) for backbone rotation of G83. a) active ERK2, b) inactive ERK2, and c) ERK2 mutant G83A.

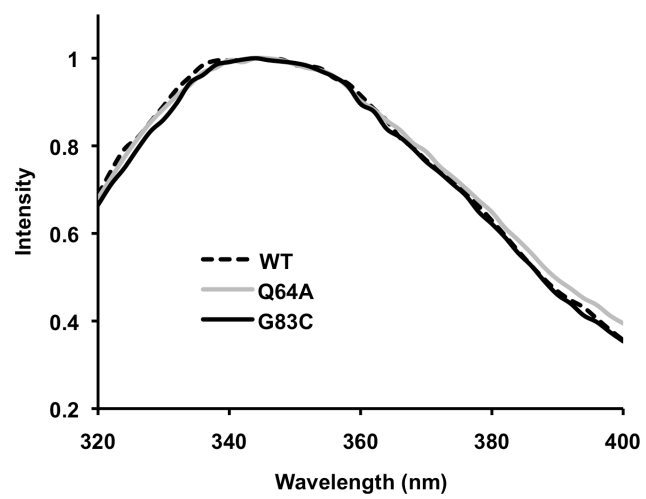


Figure S6. Fluorescence spectra of ERK2 wild type and mutants.

Movie M1. Quasi-harmonic mode 16 of the ERK2 inactive state showing hinge motion.

Movie M2. Quasi-harmonic mode 13 of the ERK2 active state showing hinge motion.

Movie M3. Quasi-harmonic mode 14 of the ERK2 active state showing twisting motion.

Movie M4. TMD transition pathway of ERK2 activation. The movie starts at the inactive and ends at the active state.