Support information

Q9WUL6 M3K14_MOUSE Q99558 M3K14_HUMAN	1 1	MAVMEVACPGTPGSAVGQQKELAKAKEKTQSLGKKQSCIFKLEAVEKSPVFCGKWEILND MAVMEMACPGAPGSAVGQQKELPKAKEKTPPLGKKQSSVYKLEAVEKSPVFCGKWEILND *****:****:************	60 60
Q9WUL6 M3K14 MOUSE Q99558 M3K14 HUMAN	61 61	VITKGTAKDGSEGGPPAISIIAQAECENSQEFSPTFSERIFIAGSQQYSQSESLDQIPNN VITKGTAKEGSEAGPAAISIIAQAECENSQEFSPTFSERIFIAGSKQYSQSESLDQIPNN **************	120 120
Q9WUL6 M3K14_MOUSE Q99558 M3K14_HUMAN	121 121	VAHATEGKMARVCRRGKRHGKARKKRRKKRSKSLAQAGVALAKPLPRTPEQESCTIPVQE VAHATEGKMARVCWKGKRRSKARKKRKKKSSKSLAHAGVALAKPLPRTPEQESCTIPVQE ************************************	180 180
Q9WUL6 M3K14 MOUSE Q99558 M3K14 HUMAN	181 181	DESPLGNLYARNVSQFTKPLGGPGLGHLCFKKQDEGLRPVLPRPELHKLISPLQCLNHVW DESPLGAPYVRNTPQFTKPLKEPGLGQLCFKQLGEGLRPALPRSELHKLISPLQCLNHVW ****** *.**. ****** ****** *****	240 240
Q9WUL6 M3K14 MOUSE Q99558 M3K14 HUMAN	241 241	KLHHPQATGPRPHPTHPFPYSGMPHPFPFYPLEPWKPYMLDSAVLDKLAGVSGQRPLPGP KLHHPQDGGPLPLPTHPFPYSRLPHPFPFHPLQPWKPHPLES-FLGKLACVDSQKPLPD- ****** ** * ******** :******:**:**: *:* .*.****	300 298
Q9WUL6 M3K14 MOUSE Q99558 M3K14 HUMAN	301 299	PHLSQLAHGDSQKPLPGPHLESSCPSRGALEKVPVEEYLVHALQGSVSSGQAHSLASLAK PHLSKLACVDSPKPLPGPHLEPSCLSRGAHEKFSVEEYLVHALQGSVSSGQAHSLTSLAK ****:** ** ********* ** **** **. ********	360 358
Q9WUL6 M3K14 MOUSE Q99558 M3K14 HUMAN	361 359	TWSSGSAKLQRLGPETEDNEGVLLTEKLKPVDYEYREEVHWMTHQPRVGRGSFGEVHRMK TWAARGSRSREPSPKTEDNEGVLLTEKLKPVDYEYREEVHWATHQLRLGRGSFGEVHRME **:: .:: :*:************************	420 418
Q9WUL6 M3K14_MOUSE Q99558 M3K14_HUMAN	421 419	DKQTGFQCAVKKVRLEVFRVEELVACAGLSSPRIVPLYGAVREGPWVNIFMELLEGGSLG DKQTGFQCAVKKVRLEVFRAEELMACAGLTSPRIVPLYGAVREGPWVNIFMELLEGGSLG **********************************	480 478
Q9WUL6 M3K14 MOUSE Q99558 M3K14 HUMAN	481 479	QLIKQMGCLPEDRALYYLGQALEGLEYLHTRRILHGDVKADNVLLSSDGSRAALCDFGHA QLVKEQGCLPEDRALYYLGQALEGLEYLHSRRILHGDVKADNVLLSSDGSHAALCDFGHA **:*: ********************************	540 538
Q9WUL6 M3K14_MOUSE Q99558 M3K14_HUMAN	541 539	LCLQPDGLGKSLLTGDYIPGTETHMAPEVVMGKPCDAKVDIWSSCCMMLHMLNGCHPWTQ VCLQPDGLGKSLLTGDYIPGTETHMAPEVVLGRSCDAKVDVWSSCCMMLHMLNGCHPWTQ :************************************	600 598
Q9WUL6 M3K14_MOUSE Q99558 M3K14_HUMAN	601 599	YFRGPLCLKIASEPPPIREIPPSCAPLTAQAIQEGLRKEPVHRASAMELRRKVGKALQEV FFRGPLCLKIASEPPPVREIPPSCAPLTAQAIQEGLRKEPIHRVSAAELGGKVNRALQQV :***********************************	660 658
Q9WUL6 M3K14 MOUSE Q99558 M3K14 HUMAN	661 659	GGLKSPWKGEYKEPRPPPQDQATCHQTLPTPPRENPPAKANTDGAPEPQPPLP GGLKSPWRGEYKEPRHPPPNQANYHQTLHAQPRELSPRAPGPRPAEETTGRAPKLQPPLP *******:****** ** :**. **** : *** * *: .*. **: *****	713 718
Q9WUL6 M3K14_MOUSE Q99558 M3K14_HUMAN	714 719	PEPPEPSKAPALNLSKEESGTWEPLPLSSLDPATAKGPSFPDRRATLPELELQQLEIELF PEPPEPNKSPPLTLSKEESGMWEPLPLSSLEPAPARNPSSPERKATVPEQELQQLEIELF ******.*.*.*.*.*.*******	773 778
Q9WUL6 M3K14 MOUSE Q99558 M3K14 HUMAN	774 779	LNSLSQPFSLEEQEQILSCLSIDSLSLSDDSEKNPSKASQSSRDTLSSGVHSWNSQAEAR LNSLSQPFSLEEQEQILSCLSIDSLSLSDDSEKNPSKASQSSRDTLSSGVHSWSSQAEAR ***********************************	833 838
Q9WUL6 M3K14 MOUSE Q99558 M3K14 HUMAN	834 839	TCSCSTALARGRPTDIPSYFNGVKVQIQSLNGEHLHIREFHRVKVGDIATGISSQIPATA SSSWNMVLARGRPTDTPSYFNGVKVQIQSLNGEHLHIREFHRVKVGDIATGISSQIPAAA *********	893 898
Q9WUL6 M3K14_MOUSE Q99558 M3K14_HUMAN	894 899	FSLVTKDGQPVCYDMEVPDSGIDLQCTLAPDGSFAWTWRVKHGQLENRP FSLVTKDGQPVRYDMEVPDSGIDLQCTLAPDGSFAWSWRVKHGQLENRP **********	942 947
Identical positions	798		
Identity	84.08	9%	
Similar positions	86		
Program	CLUS	TALO	

Figure S1: UniProt Align results of human and mouse NIK protein sequence.

Q9WUL6 M3K14 MOUSE 096013 PAK4 HUMAN	1 1	MAVMEVACPGTPGSAVGQQKELAKAKEKTQSLGKKQSCIFKLEAVEKSPVFCGKWEILND MFGKRKKRVEISAP-SNFEHRVHTGFDQH-EQKFTGLPRQWQSLIE .*::*:: :: * :* : :*: *:	60 44
Q9WUL6 M3K14 MOUSE 096013 PAK4_HUMAN	61 45	VITKGTAKDGSEGGPPAISIIAQAECENSQEFSPTFSERIF ESARRPKPLVDPACITSIQPGAPKTIVRGSKGAKDGALTLLLDEFENMSVTRSNSLR :: *: *: *: * * * * * * *:	101 101
Q9WUL6 M3K14 MOUSE 096013 PAK4 HUMAN	102 102	IAGSQQYSQSESLDQIPNNVAHATEGKMARVCRRGKRHGKARKKRRKKRSK RDSPPPPARARQENGMPEEPATTARGGPGKAGSRGRFAGHSEAGGGSGDRRRAGPEKRPK . ::: : :*:: * ::.* .:. **: *. *. *: *: *: *: *:	152 161
Q9WUL6 M3K14 MOUSE 096013 PAK4 HUMAN	153 162	SLAQAGVALAKPLPRTPEQESCTIPVQEDESPLGNLYARNVSQFTKPLGGPGLGHLCFKK SSREGSGGPQESSRDKRPLSGPDVGTPQPAG * :: *: .:**.**.:*	212 192
Q9WUL6 M3K14 MOUSE 096013 PAK4_HUMAN	213 193	QDEGLRPVLPRPELHKLISPLQCLNHVWKLHHPQATGPRPHPTHPFPYSGMPHPFPFYPL LASGAKLAAGRPFNTYPR .*: .:** **	272 210
Q9WUL6 M3K14 MOUSE 096013 PAK4_HUMAN	273 211	EPWKPYMLDSAVLDKLAGVSGQRPLPGPPHLSQLAHGDSQKPLPGPHLESSCPSRGALEK ADTDHPSRGAQGEPHDVAPNGPSAGGLAIPQSSSSSSRPPTRARGAPSPGVLGP : .*: * * : * **: *** ***	332 264
Q9WUL6 M3K14 MOUSE 096013 PAK4 HUMAN	333 265	VPVEEYLVHALQGSVSSGQAHSLASLAKTWSSGSAKLQRLGPETEDNEGVLL-TEKLK HASEPQLAPPACTPAAPAVPGPPGPRSPQREPQRVSHEQFRAA * *. : : * **.: :.* : *:::	389 307
Q9WUL6 M3K14 MOUSE 096013 PAK4 HUMAN	390 308	-PVDYEYREEVHWMTHQPRVGRGSFGEVHRMKDKQTGFQCAVKKVRLEVFRVEEL LQLVVDPGDPRSYLDNFIKIGEGSTGIVCIATVRSSGKLVAVKKMDLRKQQRRELLFNEV : : : : :: :: :: :: :: :: * ***: *. ::: ****: *.	443 367
Q9WUL6 M3K14 MOUSE 096013 PAK4_HUMAN	444 368	VACAGLSSPRIVPLYGAVREGPWVNIFMELLEGGSLGQLIKQMGCLPEDRALYYLGQALE VIMRDYQHENVVEMYNSYLVGDELWVVMEFLEGGALTDIVTHTRMNEEQIAA-VCLAVLQ *:* :*.: * ::.**:****** ::::: *: *: *: *:	503 426
Q9WUL6 M3K14 MOUSE 096013 PAK4_HUMAN	504 427	GLEYLHTRRILHGDVKADNVLLSSDGSRAALCDFGHALCLQPDGLGKSLLTGDYIPGTET ALSVLHAQGVIHRDIKSDSILLTHDGR-VKLSDFGFCAQVSKEVPRRKSLVGTPY .*. **:: ::* *:*:*:*: ** . *.*** . :. *: :: **	563 480
Q9WUL6 M3K14 MOUSE 096013 PAK4 HUMAN	564 481	HMAPEVVMGKPCDAKVDIWSSCCMMLHMLNGCHPWTQYFRGPLCLKIAS-EPPPIREIPP WMAPELISRLPYGPEVDIWSLGIMVIEMVDGEPPYFNEPPLKAMKMIRDNLPPRLKNLHK ****:: * . :***** *::.*:: * : * . ** ::::	622 540
Q9WUL6 M3K14 MOUSE 096013 PAK4 HUMAN	623 541	SCAPLTAQAIQEGLRKEPVHRASAMELRRKVGKALQEVGGLKSPWKGEYKEPRPPPQDQA -VSPSLKGFLDRLLVRDPAQRATAAELLKHPFLAKAGPPASIV :* ::. * ::*.:**:* ** : *: *: **	682 582
Q9WUL6 M3K14 MOUSE 096013 PAK4 HUMAN	683 583	TCHQTLPTPPRENPPAKANTDGAPEPQPPLPPEPPEPSKAPALNLSKEESGTWEPLPLSS PLMRQNRTR	742 591
Q9WUL6 M3K14 MOUSE 096013 PAK4_HUMAN	743 592	LDPATAKGPSFPDRRATLPELELQQLEIELFLNSLSQPFSLEEQEQILSCLSIDSLSLSD	802 591
Q9WUL6 M3K14 MOUSE O96013 PAK4 HUMAN	803 592	DSEKNPSKASQSSRDTLSSGVHSWNSQAEARTCSCSTALARGRPTDIPSYFNGVKVQIQS	862 591
Q9WUL6 M3K14 MOUSE 096013 PAK4 HUMAN	863 592	LNGEHLHIREFHRVKVGDIATGISSQIPATAFSLVTKDGQPVCYDMEVPDSGIDLQCTLA	922 591
Q9WUL6 M3K14 MOUSE 096013 PAK4 HUMAN	923 592	PDGSFAWTWRVKHGQLENRP	942 591
Identical positions	129		
Identity	13.16	3%	
Similar positions	181		
Program	CLUS	TALO	

Figure S2: UniProt Align results of human PAK4 and mouse NIK protein sequence.



Figure S3: (A) Timeline of protein-ligand contacts of NIK with compound 6 (B) Ligand torsion profile of compound 6 in NIK. It summarized the conformational evolution of every rotatable bond in the ligand throughout the simulation trajectory (C) Timeline of protein-ligand contacts of NIK with compound 4 (D) Ligand torsion profile of compound 4 in NIK.



Figure S4: (A) Timeline of protein-ligand contacts of PAK4 with compound **6 (B)** Ligand torsion profile of compound **6** in PAK4. It summarized the conformational evolution of every rotatable bond in the ligand throughout the simulation trajectory **(C)** Timeline of protein-ligand contacts of PAK4 with compound **4 (D)** Ligand torsion profile of compound **4** in PAK4.



Figure S5: (A) (B) (C) (D) Molecular surface area, solvent accessible surface area, polar surface area ligand properties of complexes in the simulation

NIK/compound 6



Figure S6: Ligand PMSF and protein secondary structure changing plots of NIK/compound 6 complex during Desmond MD simulations.

NIK/compound 4



Figure S7: Ligand RMSF and protein secondary structure changing plots of NIK/compound 4 complex during Desmond MD simulations.

PAK4/compound 4



Figure S8: Ligand RMSF and protein secondary structure changing plots of PAK4/compound 4 complex during Desmond MD simulations.

PAK4/compound 6



Figure S9: Ligand RMSF and protein secondary structure changing plots of PAK4/compound 6 complex during Desmond MD simulations.



Figure S10: Illustration of solvent accessible surface of ligand-receptor complexes that extracted from the last frame of MD results. The surface was colored with blue and green corresponding to solvent exposing degree of receptor atoms in binding pockets. (A) NIK protein (yellow ribbon) complex with compound 6 (orange stick). (B) NIK protein complex with compound 4 (blue stick). (C) PAK4 protein (blue ribbon) complex with compound 4. (D) PAK4 protein complex with complex with compound



Figure S11: RMSD and RMSF plots of four complexes in Amber MD

Distance/ Å	Identity (%)	Similarity (%)	Homology (%)
3	100	100	100
4	98.3333	100	100
5	98.4615	100	100
6	95.122	98.7805	98.7805
7	92.6316	97.8947	96.8421
8	92.3077	98.0769	97.1154
9	90.8333	96.6667	96.6667
10	91.9118	97.0588	97.0588

Table S1: Distance-similarity analysis of human NIK and mouse NIK protein binding pocket

Distance/ Å	Identity (%)	Similarity (%)	Homology (%)
3	53.8462	71.7949	69.2308
4	46.6667	65	61.6667
5	43.0769	61.5385	60
6	39.0244	56.0976	59.7561
7	35.7895	52.6316	57.8947
8	33.6538	50	55.7692
9	30.8333	47.5	51.6667
10	30.8824	47.7941	50.7353

Table S2: Distance-similarity Analysis of human PAK4 and mouse NIK protein binding pocket

Entry	Complex	HOMO (eV)	LUMO (eV)	HOMO-LUMO
	Complex			energy gap (eV)
1	NIK/Compound 6	-0.1924	-0.0477	0.1447
2	NIK/Compound 4	-0.3269	-0.1883	0.1386
3	PAK4/Compound 6	-0.1896	-0.0539	0.1357
4	PAK4/Compound 4	-0.1898	-0.0478	0.1420

Table S3: Ligand single point energy results of QM/MM minimized complexes