

Supplemental figure legends.

Supplemental Figure S1. Purity of the B-Raf and KSR1 proteins. The purity for each protein was assessed on a silver stained 4-12% SDS-PAGE gel.

Supplemental Figure S2. Analysis of the nucleotide status and activity of kinases B-Raf^{WT}, B-Raf^{V600E} and pseudokinase KSR1. **A)** RP-HPLC chromatograms of nucleotide standards and nucleotides extracted from B-Raf (wild-type and V600E mutant) and KSR1. A mixture of ADP and ATP standards, B-Raf^{WT}, B-Raf^{V600E} or KSR1 without (blue profile) or with pre-incubation (red profile) with ATP followed by dialysis to remove excess ATP and then heating to remove bound ATP or ADP were resolved by HPLC. No ATP peak was detected in all samples prior to pre-incubation with ATP, indicating that all of these samples were devoid of nucleotides. Upon pre-incubation with ATP, an ADP peak was detected in the B-Raf^{WT} and B-Raf^{V600E} samples, and an ATP peak in the KSR1 sample, suggesting that all proteins bind ATP, and that B-Raf, but not KSR1, can convert ATP to ADP. **B)** Characterization of B-Raf^{WT} and B-Raf^{V600E} in the ADP-Glo kinase assay. B-Raf^{WT} and B-Raf^{V600E} were pre-incubated with the indicated inhibitors (1 nmol) or vehicle DMSO (control) before the addition of ATP (10 nmol) for a varying time in a reaction mixture containing 10mM MgCl₂ in TEAB buffer pH 8. The data are means ± standard deviation from three independent experiments. Bars with different letters or without letters indicate statistical significant differences, according to Student's *t*-tests (*p*<0.05).

Supplemental Figure S3. Sequence coverage of B-Raf^{WT} by our limited proteolysis combined with stable isotope labelling. Peptides identified upon limited proteolysis and full digestion are underlined, with a different color for the two conditions tested. In this example,

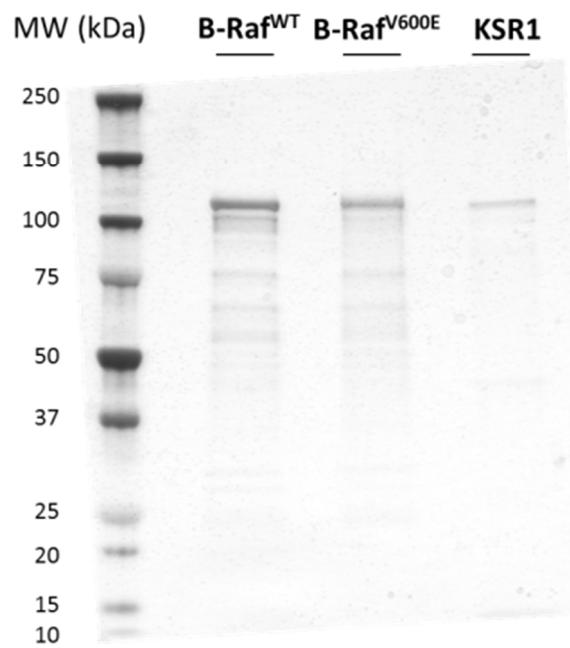
sequence coverages of 30% and 32% for the limited proteolysis and full digestion condition respectively were obtained, with a total sequence coverage of 41%. The Ras binding domain (RBD) and kinase domain (KD) region are highlighted. The example reported here for B-Raf^{WT} pre-incubated with vehicle DMSO is representative of the other samples.

Supplemental Figure S4. Conformational changes in B-Raf kinases induced by small molecule binding. **A)** Cartoon representation of the RBD and KD of B-Raf^{WT} and B-Raf^{V600E} to illustrate the changes in abundance of peptides obtained in the limited proteolysis experiments of B-Raf complexed with ATP over time. The detected peptides are coloured as follows: brown: SPQKPIVR (aa 151-158), blue: VFLPNK (aa 159-164), green: TVVPAR (aa 167-172), magenta: DSLKK (aa 179-183), cyan: ALMMR (aa 184-188), light brown: DSSDDWEIPDGQITVGQR (aa 445-462), dark blue: IGSGFGTVYK (aa 463-472), pink: WHGDVAVK (aa 476-483), dark green: LIDIAR (aa 553-558), red: QTAQGMDYLHAK (aa 559-570), light blue: SIIHRDLK (aa 571-578), yellow: SNNIFLHEDLTVK (aa 579-591), purple: IGDFGLATVK/ IGDFGLATEK (aa 592-601), orange: GYLSPDLSK (aa 672-680). The PDB-entry 3NY5 (ref to be published) and the 3OG7 (1) were used to visualize the RBD and the KD, respectively. **B)** Examples of the double charged ion isotope-peak pair corresponding to tryptic peptides of the RBD (SPQKPIVR, aa 151-158 and VFLPNK, aa 159-164) and KD (IGSGSFGTVYK, 463-473 and IGDFGLATEK, aa 592-601) domain modified by a light or heavy label ($\Delta m/z = 4$) from B-Raf^{WT} alone (control) or in complex with vemurafenib or sorafenib inhibitor. **C)** Superposition of B-Raf^{WT} with vemurafenib (PDB-entry 1UWH, (2)) and B-Raf^{V600E} with sorafenib (PDB-entry 3OG7, (1)) bound. A. Overall view. B. Focus on the N-lobe. As can be seen, several loops of the N-lobe adopt a different conformation upon vemurafenib (active conformation) or sorafenib (inactive

conformation) binding. Images were generated in Pymol (The PyMOL Molecular Graphics System, Version 1.5.0.4, Schrödinger, LLC).

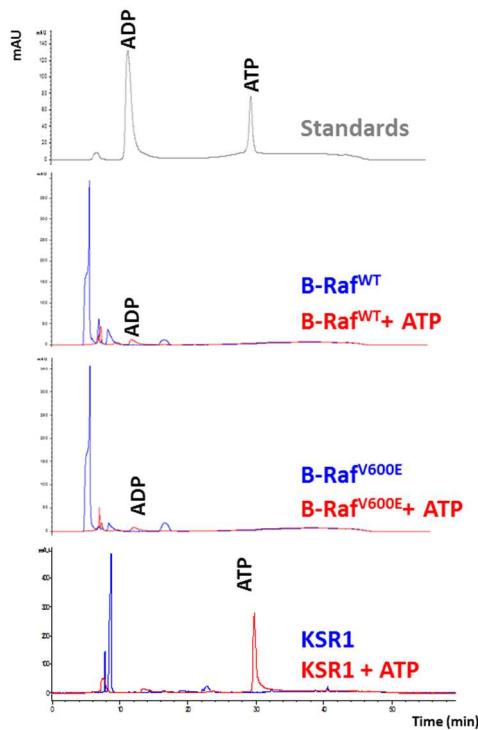
Figure S5. HDX-MS analyses on binding of B-Raf^{WT} to different small molecules and inhibitors. Deuterium uptake curves for one peptide (FMGYSTKPQLA, aa 516-526) of the B-Raf^{WT} KD domain. A reduced deuteration exchange upon binding of ATP, AMP-PNP, vemurafenib and sorafenib, was shown for several peptides of the KD (of which 516-526 here shown is an example), thus indicating a stabilizing effect on this domain, while no alterations in deuterium incorporation were observed for the peptides belonging to other B-Raf regions (Supplemental Table S2).

Supplemental Figure S1

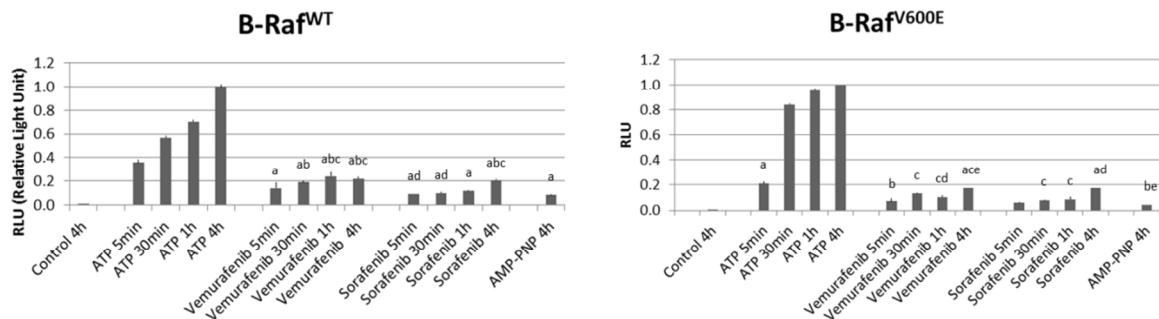


Supplemental Figure S2

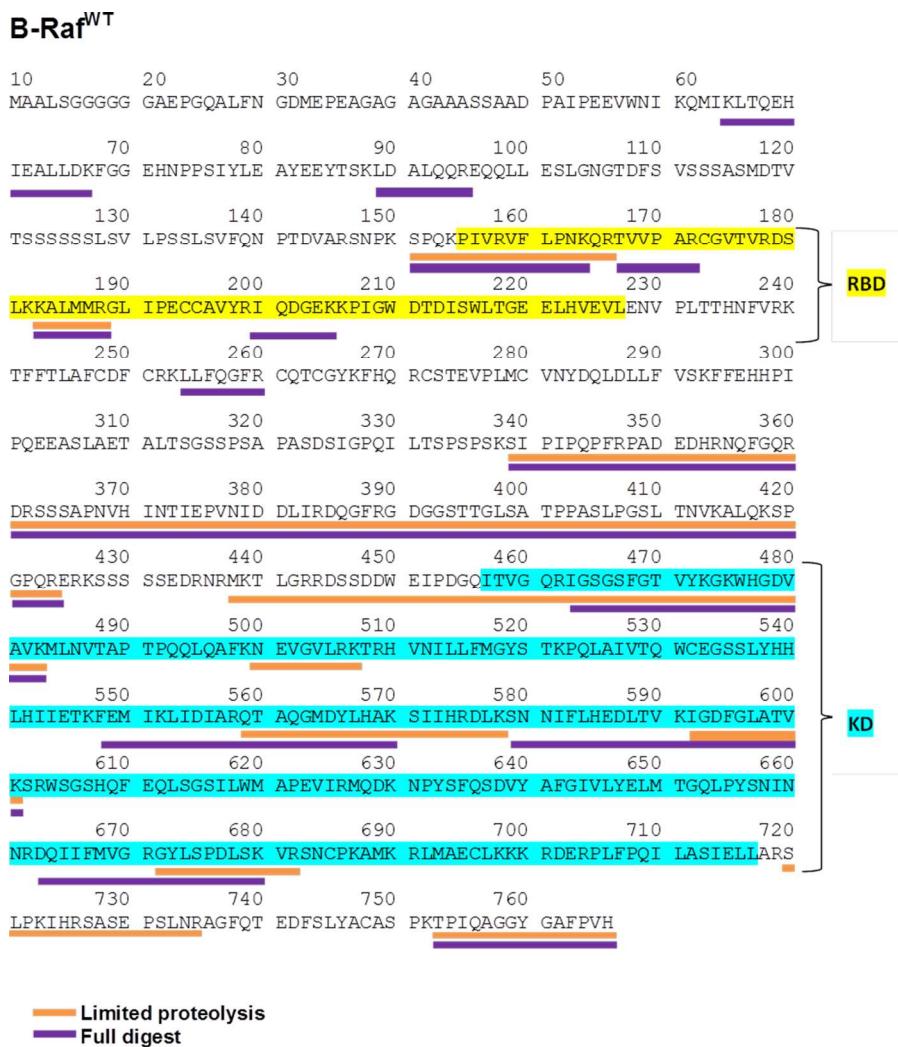
A



B

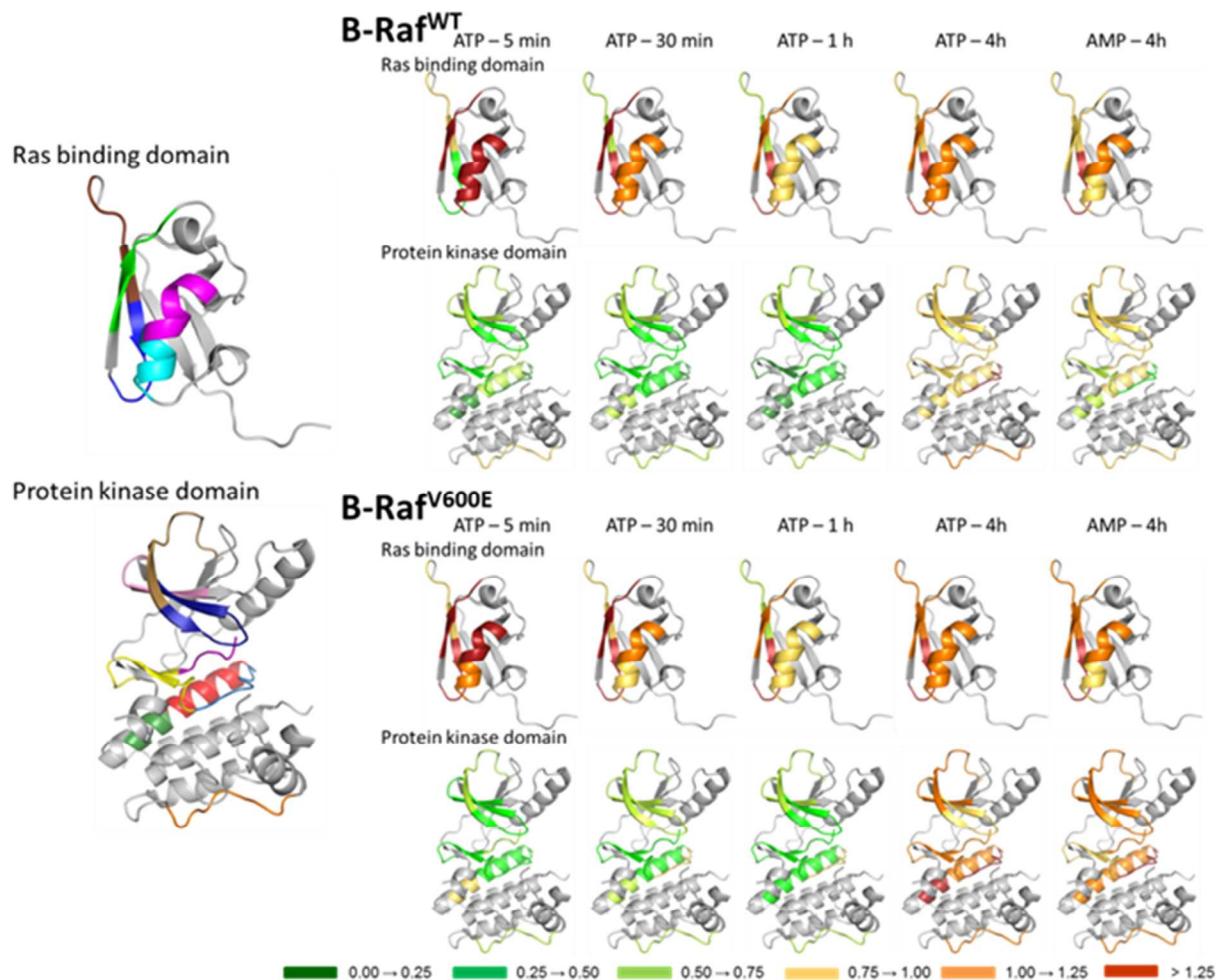


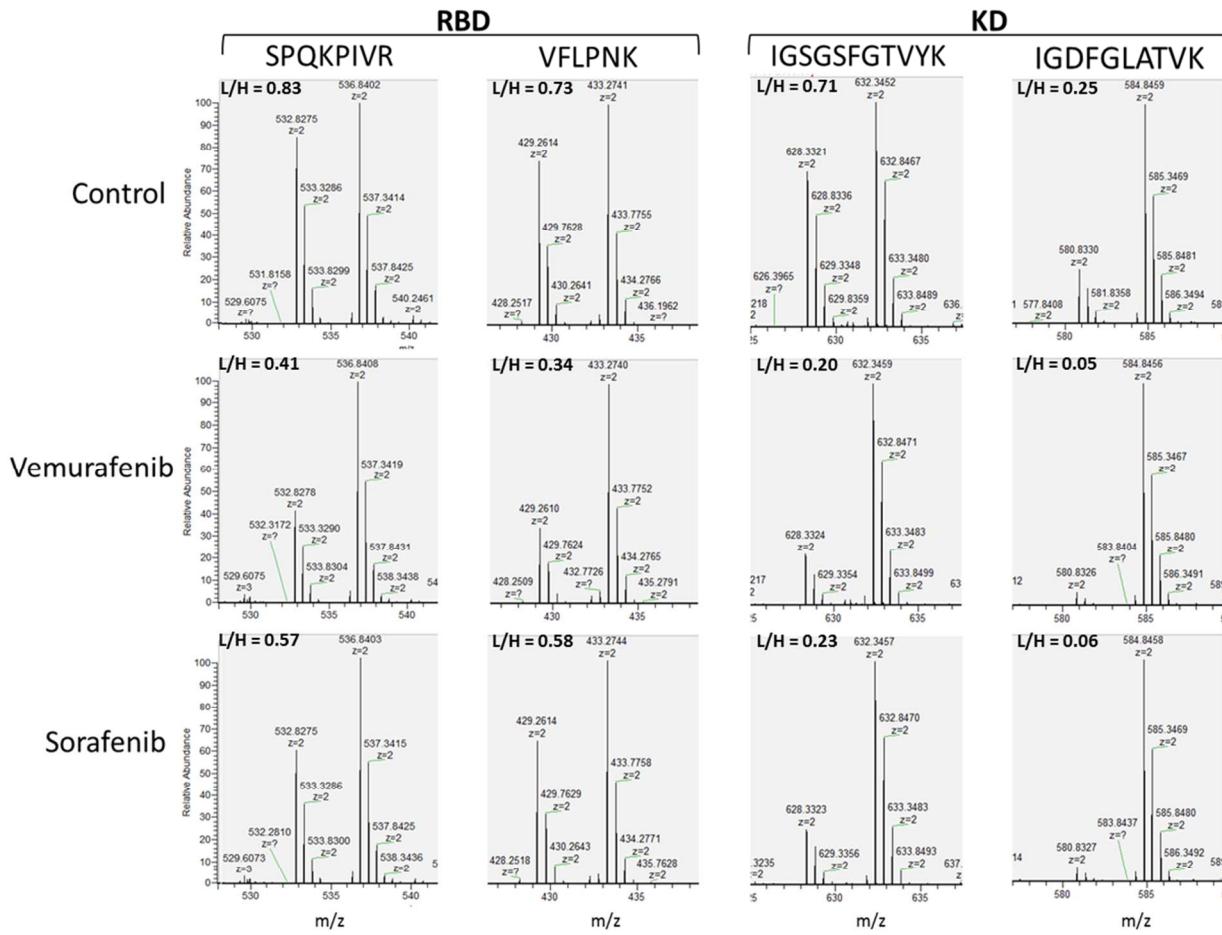
Supplemental Figure S3



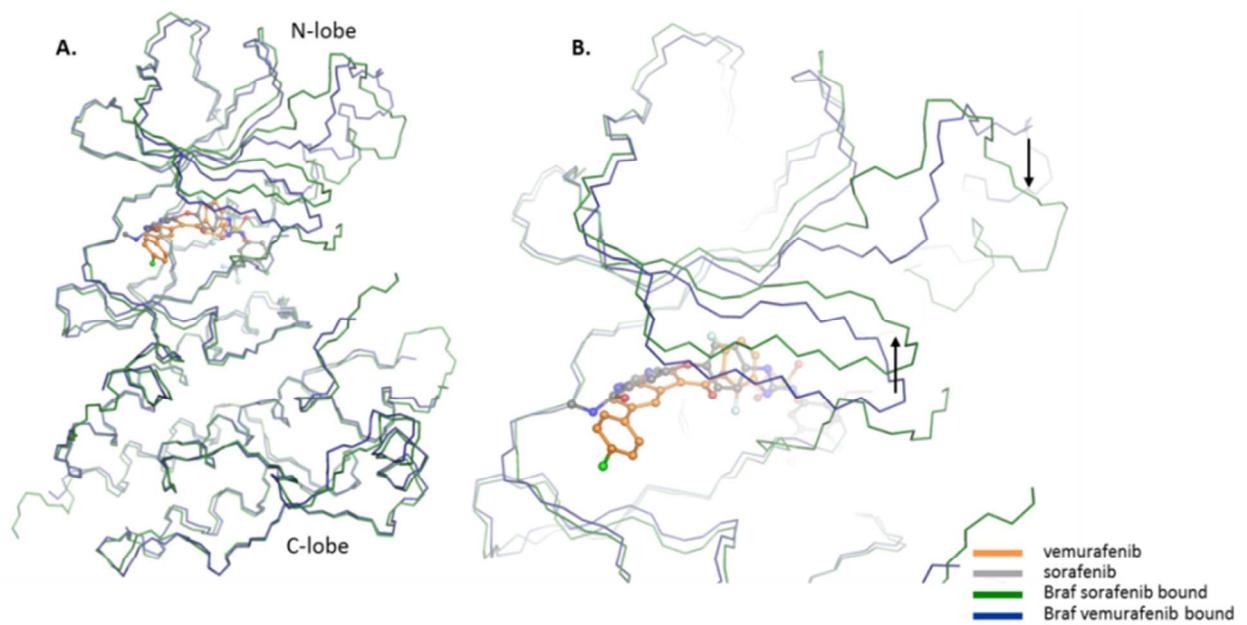
Supplemental Figure S4

A

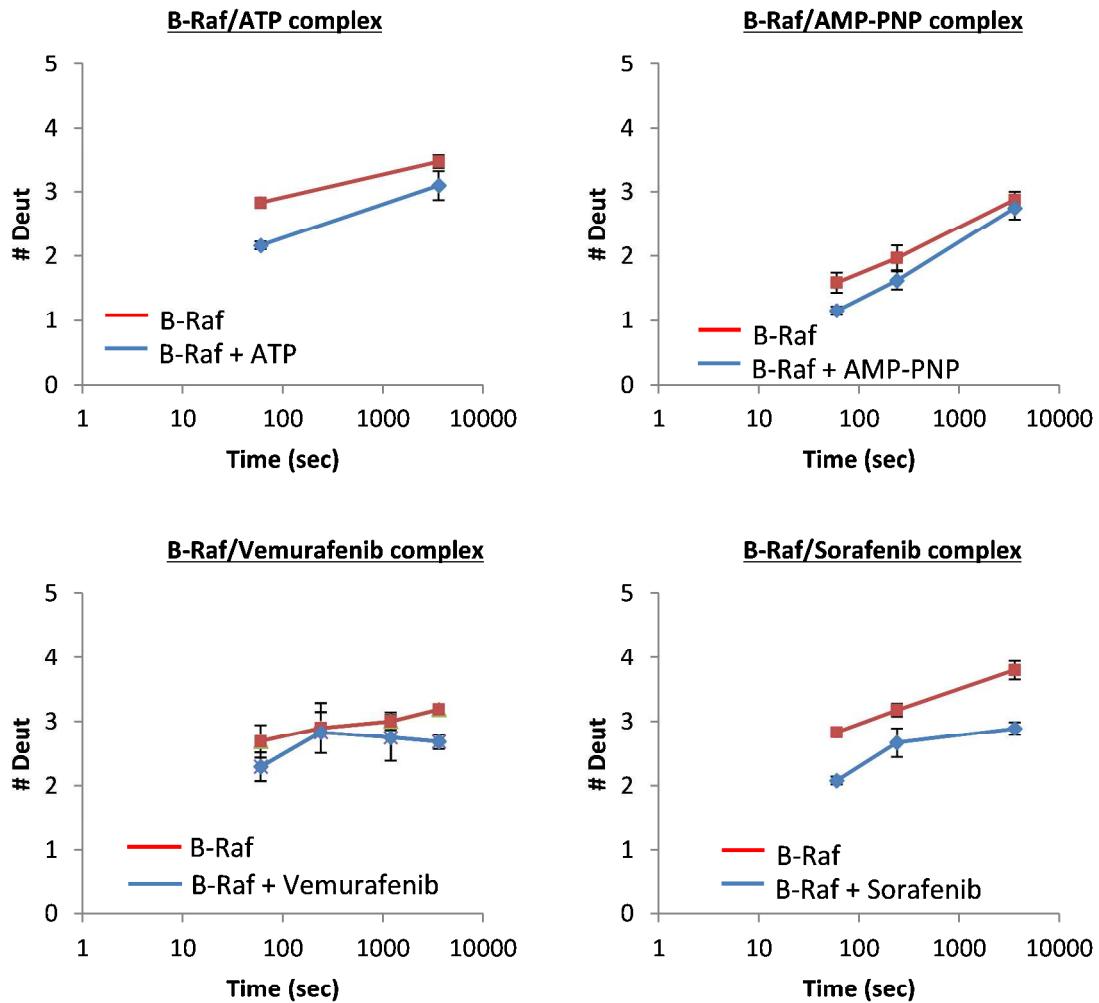


B

C



Supplemental Figure S5



Supplemental Table S1. Conformational changes in B-Raf kinases induced by small molecule binding. B-Raf^{WT} was incubated with vehicle 1% DMSO, 10 nmol ADP, ATP or AMP-PNP for 4 h at 30°C with or without 1 mM MgCl₂. The table shows the relative abundances of B-Raf^{WT} peptides released upon limited proteolysis and quantified by stable isotope labelling complexed with different nucleotides in the absence or presence of MgCl₂. The abundance is expressed with a colour scale ranging from red (higher amount) to green (lower amount). The values indicate the average of three independent experiments, normalized for the control ± the standard deviation. The values underlined and in bold typeface indicate statistically significant differences between control and small molecule treated samples according to Student's *t*-tests (*p*<0.05) calculated on log₂ (ratio) values.

Region	Start	Sequence	No Mg ²⁺			+ Mg ²⁺		
			ADP	ATP	AMP-PNP	ADP	ATP	AMP-PNP
RBD	151	SPQKPIVR	1.31 ±0.13	0.74 ±0.18	0.47 ±0.04	0.99 ±0.38	0.87 ±0.06	1.09 ±0.36
	159	VFLPNK	1.20 ±0.21	0.68 ±0.13	0.44 ±0.03	1.90 ±1.05	1.32 ±0.38	1.53 ±0.87
	167	TVVPAR	1.57 ±0.53	0.89 ±0.52	0.81 ±0.09	1.39 ±0.48	1.71 ±0.97	4.41 ±5.40
	179	DSLKK	1.08 ±0.11	0.72 ±0.18	0.29 ±0.06	0.96 ±0.22	1.11 ±0.20	0.86 ±0.24
	339	SIPIPQPFRPADEDHR	1.20 ±0.38	0.62 ±0.17	0.38 ±0.03	1.36 ±0.17	0.96 ±0.11	1.00 ±0.40
	355	NQFGQQR	1.04 ±0.16	0.86 ±0.27	0.59 ±0.16	1.20 ±0.20	1.34 ±0.30	1.06 ±0.23
	390	GDGGSTTGLSATPPASLPGSLTNVK	1.24 ±0.07	1.16 ±0.50	0.51 ±0.08	1.03 ±0.26	1.17 ±0.45	1.42 ±0.37
	427	KSSSSSEDR	2.18 ±1.15	1.29 ±0.48	0.92 ±0.17	1.39 ±0.31	1.21 ±0.38	1.18 ±0.07
KD	445	DSSDDWEIPDGQITVGQR	1.16 ±0.24	0.88 ±0.05	0.65 ±0.10	1.11 ±0.06	0.97 ±0.09	0.88 ±0.16
	463	IGSGSFGTVYK	1.19 ±0.21	0.81 ±0.18	0.56 ±0.04	1.13 ±0.15	0.85 ±0.24	0.97 ±0.24
	474	GKWHGDVAVK	1.00 ±0.01	0.81 ±0.10	0.57 ±0.08	1.19 ±0.24	1.15 ±0.31	1.06 ±0.23
	500	NEVGVLR	0.60 ±0.41	0.68 ±0.36	0.95 ±0.54	1.40 ±0.84	1.47 ±0.91	1.10 ±0.43
	553	LIDRAR	0.97 ±0.15	0.73 ±0.29	0.22 ±0.10	4.00 ±3.78	2.97 ±1.96	1.86 ±0.67
	559	QTAQGMDYLHAK	1.15 ±0.28	0.95 ±0.27	0.68 ±0.12	1.38 ±0.48	1.28 ±0.50	1.27 ±0.63
	592	IGDFGLATVK	1.01 ±0.82	0.93 ±0.78	0.58 ±0.36	1.13 ±0.27	1.20 ±0.49	1.10 ±0.40
	663	DQIIFMVGR	0.69 ±0.45	0.24 ±0.32	0.08 ±0.07	1.01 ±0.30	1.70 ±1.06	1.76 ±1.04
	672	GYLSPDLSK	0.96 ±0.09	0.66 ±0.17	0.36 ±0.09	1.35 ±0.57	1.41 ±0.67	1.19 ±0.55
	727	SASEPSLNR	0.94 ±0.04	0.78 ±0.12	0.52 ±0.06	1.15 ±0.15	1.23 ±0.25	1.09 ±0.21
	753	TPIQAGGYGAfpvh	1.55 ±0.59	0.97 ±0.28	0.82 ±0.25	1.11 ±0.29	1.21 ±0.28	1.06 ±0.29

Supplemental Table S2. HDX-MS analyses on binding of B-Raf^{WT} to different small molecules and inhibitors. B-Raf was incubated with vehicle 1% DMSO, 17 nmol of ATP and AMP-PNP and 170 pmol of vemurafenib and sorafenib for 4 h at 30°C and analyzed to HDX-MS. The table reports the average of three measurements with the standard deviation shown below. Values in bold represent statistically significant differences in deuterium incorporation ($p<0.05$). Relative deuteration (#D) was measured at different incubation times (1, 4 or 60 min) in three independent measurements. The main B-Raf^{WT} regions are indicated: Ras binding domain (RBD, aa 155-227) and kinase domain (KD, aa 457-717).

Start	End	Sequence	B-Raf			B-Raf + ATP			B-Raf			B-Raf + AMP-PNP			B-Raf			B-Raf + Vemurafenib			B-Raf			BR-Raf + Sorafenib				
			1min	4min	60min	1min	4min	60min	1min	4min	60min	1min	4min	60min	1min	4min	60min	1min	4min	60min	1min	4min	60min	1min	4min	60min		
RDB	144	173 VARSNPKSPQKPV/RVFLPNKQRTV/PARC										7.63	9.66	12.50	7.79	9.51	12.56											
	158	173 RVFLPNKQRTV/PARC										0.04	0.38	0.39	0.21	0.17	0.45											
	174	188 GVTVRDSLKKALM										0.54	0.38	0.18	0.33	0.36	0.26											
	187	195 MRGLIPECC	0.71	1.91	0.49	1.95						0.06	0.08	0.09	0.07	0.07	0.08											
	197	222 VYRQDGKPKGWDTSWLTGEEL	7.08	10.19	6.29	9.54	3.67	4.82	7.84	3.52	4.77	7.60	5.04	6.85	0.27	5.57	6.77	9.20	7.09	8.45	11.32	6.84	8.27	10.98				
	214	222 ISWLGEEL	0.07	0.08	0.04	0.04	0.09	0.15	0.17	0.08	0.02	0.13	0.11	0.03	0.08	0.10	0.05	0.05	0.20	0.09	0.09	0.06	0.04	0.07	0.16			
223	246 HVEVLENVPLTHNFRVKTFITLA	7.68	6.65			4.80	5.81	8.47	4.52	5.55	8.11	7.13	9.31	10.09	7.18	9.28	10.67	7.71	9.16	11.52	7.40	9.23	11.50					
	251	258 CRKLLFQG	0.42	0.26								1.13	1.44	1.90	1.11	1.39	1.83											
	290	306 FVSKFFEEHHPIPQEAEASL	0.01	0.04			4.01	4.24	4.59	3.96	4.44	4.67	0.01	0.03	0.03	0.06	0.05	0.03										
	290	307 FVSKFFEEHHPIPQEAEASL			0.12	0.02	0.12	0.06	0.07	0.02																		
	295	307 FEHHPIPQEAEASL	3.86	3.02	3.64	3.07	3.94	3.97	3.96	3.66	4.01	4.02	3.78	3.76	3.73	3.76	3.72	3.69	3.86	3.74	3.87	3.75	3.61	3.54				
	383	398 IRDQQFRGDDGGSTTGL	6.94	6.77	6.64	6.99	0.05	0.18	0.07	0.11	0.07	0.17	0.03	0.02	0.06	0.03	0.04	0.06	0.02	0.08	0.01	0.09	0.05	0.08	0.09			
383	398 IRDQQFRGDDGGSTTGLS	0.01	0.05	0.18	0.07							6.09	6.14	6.07	6.13	6.13	5.97	6.94	6.84	7.03	6.74	6.45	6.98					
	395	413 TTGLSATPPASLPGSLTNV	2.12	3.31	1.79	2.24	0.04	0.04	0.04	0.04	0.07	0.09	2.40	2.86	3.29	2.38	2.81	3.25	2.12	2.65	3.22	2.03	2.74	3.16				
	400	417 APPASLPGSLTNV/KALQ	8.34	7.93	8.13	8.09	7.83	7.97	8.00	7.50	8.29	8.02	0.03	0.07	0.03	0.02	0.05	0.01	0.11	0.06	0.08	0.05	0.15	0.12	0.05			
	450	466 WEIPDGQHIVGRGDSF										7.85	7.87	8.01	7.30	7.70	8.07											
	482	497 VKMLVNATPTPQLQQA										0.04	0.04	0.03	0.03	0.03	0.02											
	485	497 LNVTAPTPQLQQA	5.07	5.58	4.30	5.64	3.78	4.42	5.36	2.94	3.89	5.47	6.43	6.14	6.89	7.23	5.59	6.39	7.13	5.07	5.27	5.48	4.53	5.25	5.68			
516	526 YMGSYTKPQLA	2.83	3.47	2.16	3.10	1.59	1.97	2.87	1.15	1.62	2.74	2.69	2.60	2.90	3.19	2.29	2.82	2.68	2.83	3.18	3.80	2.08	2.66	2.89				
	517	526 YMGSYTKPQLA	0.01	0.05	0.02	0.02	0.05	0.07	0.04	0.02	0.03	0.06	0.06	0.07	0.10	0.04	0.08	0.13	0.02	0.03	0.05	0.15	0.10	0.05				
	519	526 YSTKPKQLA										1.81	2.65	1.03	1.41	2.59												
	519	526 YSTKPKQLA										1.04	1.26	1.97	0.77	1.08												
	527	550 VTQWCCEGSSLYHHLIIETKFEM	4.42	5.88	3.95	5.41	2.81	3.14	4.69	2.52	3.08	4.21	3.64	4.35	5.63	3.64	4.33	4.72	4.42	5.16	6.46	3.94	4.42	5.84				
	538	545 YHHLHIE	0.20	0.09	0.16	0.04	0.11	0.21	0.04	0.08	0.07	0.05	0.05	0.06	0.07	0.04	0.08	0.13	0.02	0.12	0.14	0.04	0.07	0.05				
KD	538	546 YHHLHIE										0.13	0.09	0.46	0.04	0.13	0.24											
	554	565 IDARQTAQGMD	4.72	5.10	0.26	0.60	0.21	0.23	0.21	0.13	0.22	0.04	0.47	0.52	0.29	0.24	0.55	0.50	4.72	4.81	5.79	0.21	0.18	0.27				
	561	581 YHLAKSIIIRDLKSNN	2.52									1.57	1.71	2.60	1.07	1.31	2.26	2.52	2.73	3.27	0.92	0.97	2.26					
	582	588 IFLHEDL										0.14	0.19	0.07	0.07	0.06	0.12	0.06	0.11	0.02	0.27	0.01						
	589	597 TVKIGDFGL	0.83									0.29	0.24	0.24	0.04	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24		
	619	627 WMAPEVIRM	2.60	3.23	1.19	2.26	1.33	1.58	2.44	0.77	0.77	1.60	1.61	2.06	3.00	1.32	1.82	3.01	2.60	2.84	3.35	1.02	1.25	2.06				
619	639 WMAPEVIRMQDNKPNYSQFDSDV	8.40	7.91	5.91	7.46							0.34	0.54	0.78	0.26	0.43	0.80	0.20	0.37	0.49	0.03	0.12	0.39					
	628	641 QDKNPYSQFDSDVY	5.05	5.25	3.48	4.49						3.77	4.23	4.61	3.22	3.68	4.21	5.05	5.26	5.87	2.96	3.65	4.48					
	647	663 YELMTQLQLYSNNNRD	5.96	6.83	4.92	6.35	4.52	4.98	6.18	4.00	4.55	5.60	4.99	5.44	6.24	4.76	5.13	6.04	5.96	6.47	7.30	4.71	5.17	6.26				
	668	678 MVGRGYSPDL	0.99	2.60	0.98	3.38	0.08	0.06	0.07	0.13	0.01	0.11	0.03	0.06	0.02	0.07	0.08	0.01	0.04	0.06	0.03	0.07	0.02	0.05				
	717	738 LARSLPKIHRSASEPSLNRAGF	8.82	8.54	8.56	8.66						5.54	7.89	7.93	7.73	7.92	7.91	8.83	8.89	9.40	8.59	8.96	9.26					
	734	742 NRAGFQTED	4.02									4.20	4.22	4.19	4.22	4.19	4.12	4.02	4.17	4.14	3.98	4.34	4.13					
743	760 FSLYACASPKTPIQAGGYGAFPVH	5.09									10.56	10.64	10.66	10.57	10.60	10.57	5.09	5.64	6.13	4.91	5.54	6.06						
	746	760 YACASPKTPIQAGGYGAFPVH	0.02									0.05	0.07	0.06	0.05	0.06	0.05	0.02	0.07	0.06	0.09	0.02	0.05					
	746	763 YACASPKTPIQAGGYGAFPVH	8.49	8.50	8.46	8.66	0.14	0.12	0.06	0.01	0.08	0.02	7.59	7.82	7.68	7.22	7.86	7.75										
	746	766 YACASPKTPIQAGGYGAFPVH	0.04	0.08	0.03	0.11						7.87	7.85	7.81	7.84	7.82	7.63											
	747	766 ACASPKTPIQAGGYGAFPVH										0.05	0.17	0.07	0.01	0.01	0.16											

p<0.01

Supplemental Table S3. Raw data of the limited proteolysis experiments performed on B-Raf and KSR1.

The protein of interest was incubated with the indicated small molecules or inhibitors for 4 h at 30°C and subjected to limited proteolysis. The tables report the raw data (before normalization to the control, averaging and conversion to log2 values) of the results presented in Tables 1(A), 2 (B), 4 (C) and S1 (D), respectively.

A

Region	Start	Sequence	Experiment 1						Experiment 2						Experiment 3					
			Control	ADP	ATP	AMP-PNP	Vemurafenib	Sorafenib	Control	ADP	ATP	AMP-PNP	Vemurafenib	Sorafenib	Control	ADP	ATP	AMP-PNP	Vemurafenib	Sorafenib
RBD	151	SPQKPNVR	0.83	0.98	0.36	0.12	0.41	0.57	0.50	0.18	0.13	0.07	0.13	0.25	0.19	0.18	0.10	0.06	0.16	0.21
	159	VFLPNPKR	0.73	0.48	0.26	0.07	0.34	0.58	0.41	0.16	0.09	0.09	0.12	0.24	0.15	0.10	0.06	0.03	0.09	0.14
	160	TVPVPRAS	0.38	0.25	0.25	0.05	0.26	0.28	0.19	0.08	0.10	0.03	0.05	0.25	0.24	0.05	0.05	0.01	0.02	0.04
	148	DDSDOWEPIGQQTIVGQR	0.40	0.31	0.14	0.04	0.13	0.24	0.08	0.06	0.06	0.03	0.08	0.20	0.16	0.01	0.01	0.01	0.01	0.01
	355	NQFGQR	2.91	2.27	1.54	0.49	1.74	2.57	1.11	1.01	0.57	0.23	0.79	0.83	0.53	0.43	0.21	0.15	0.21	0.52
	427	KSSSSEEDS	1.84	1.40	1.04	0.20	1.25	0.94	1.09	0.95	1.32	1.66	0.84	0.20	5.10	4.27	3.39	3.15	3.43	
	463	KGGSGFGTVK	0.71	0.49	0.42	0.18	0.20	0.23	0.31	0.13	0.16	0.06	0.05	0.03	0.09	0.09	0.04	0.03	0.04	0.04
	476	WHGDVAVAK	0.52	0.36	0.30	0.15	0.18	0.16	0.08	0.09	0.03	0.03	0.02	0.06	0.07	0.03	0.03	0.02	0.03	
	559	TAQAGMDYHAK	0.10	0.10	0.08	0.03	0.04	0.06	0.04	0.02	0.02	0.00	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01
	592	IQDFGLATEK	0.40	0.26	0.24	0.13	0.13	0.07	0.08	0.04	0.03	0.02	0.02	0.02	0.17	0.22	0.22	0.14	0.18	
KD	592	IQDFGLATEK	0.25	0.14	0.15	0.07	0.05	0.06	0.07	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01
	672	GYSPLDSLK	0.42	0.26	0.19	0.05	0.11	0.16	0.07	0.05	0.04	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01
	727	SASEPSLNR	2.70	2.00	1.59	0.59	2.02	1.58	1.43	1.69	1.44	1.04	1.03	1.24	1.41	0.79	0.64	1.31	1.44	
	753	TPQAGGYGAEPVH	2.54	1.72	1.97	0.97	2.16	2.13	1.41	0.68	1.13	0.37	0.79	0.44	0.44	0.47	0.27	0.22	0.41	0.46
	445	DSSDOWEPIGQQTIVGQR	5.01	4.00	4.64	3.99	3.56	3.14	2.79	3.10	2.49	2.00	2.18	2.37	6.65	4.90	5.15	3.63	3.88	3.49
	463	KGGSGFGTVK	0.15	0.16	0.10	0.10	0.09	0.05	0.06	0.06	0.04	0.03	0.03	0.02	0.17	0.23	0.09	0.07	0.06	0.09
	348	PAEDDEHR	0.62	0.56	0.42	0.23	0.69	0.90	0.30	0.09	0.14	0.12	0.26	0.39	1.00	0.58	0.28	0.25	0.50	1.00
	559	TAQAGMDYHAK	0.11	0.11	0.08	0.04	0.06	0.06	0.05	0.07	0.07	0.04	0.04	0.07	1.00	0.70	ND	0.50	1.20	
	592	IQDFGLATEK	0.11	0.11	0.08	0.04	0.06	0.06	0.05	0.07	0.07	0.04	0.04	0.07	1.00	0.70	ND	0.50	1.20	
	672	GYSPLDSLK	0.06	0.07	0.04	0.02	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.11	0.07	0.09	0.09	0.04	
	727	SASEPSLNR	0.94	0.89	0.73	0.44	1.10	0.68	0.99	0.75	0.72	0.62	1.11	0.88	2.78	1.44	1.20	0.83	1.63	2.29
	753	TPQAGGYGAEPVH	0.84	0.62	0.53	0.42	0.80	0.61	0.27	0.19	0.16	0.12	0.18	0.25	0.70	0.38	0.31	0.26	0.40	0.57

B

Region	Start	Sequence	Experiment 1						Experiment 2						Experiment 3					
			CNTRL	ATP 5min	ATP 30min	ATP 60min	ATP 4h	AMP 4h	CNTRL	ATP 5min	ATP 30min	ATP 60min	ATP 4h	AMP 4h	CNTRL	ATP 5min	ATP 30min	ATP 60min	ATP 4h	AMP 4h
RBD	56	LTDQHIEALLDK	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00
	56	SPQKPNVR	0.58	0.47	0.48	0.42	0.59	0.50	0.58	0.50	0.50	0.50	0.66	0.67	0.38	0.33	0.32	0.41	0.32	
	159	UDAR	0.04	0.02	0.00	0.00	0.00	0.00	0.06	0.02	0.02	0.00	0.04	0.04	0.00	0.09	0.09	0.14	0.14	
	167	TVPVPR	0.10	0.09	0.05	0.08	0.11	0.05	0.04	0.04	0.02	0.04	0.06	0.06	0.17	0.05	0.03	0.03	0.04	
	348	PAEDDEHR	0.75	0.87	0.59	0.26	0.90	1.08	0.01	0.00	0.00	0.00	0.00	0.00	2.30	0.77	0.56	0.28	0.90	
	559	KGGSGFGTVK	0.15	0.16	0.10	0.10	0.09	0.05	0.06	0.04	0.03	0.03	0.02	0.02	0.17	0.23	0.09	0.07	0.06	
	559	TAQAGMDYHAK	0.15	0.14	0.11	0.09	0.09	0.05	0.03	0.03	0.02	0.02	0.02	0.02	0.10	0.02	0.01	0.01	0.02	
	559	TAQAGMDYHAK	0.22	0.23	0.03	0.03	0.01	0.01	0.02	0.01	0.01	0.00	0.00	0.00	0.11	0.02	0.01	0.01	0.02	
	559	TAQAGMDYHAK	0.23	0.24	0.03	0.03	0.01	0.01	0.02	0.01	0.01	0.00	0.00	0.00	0.11	0.02	0.01	0.01	0.02	
	592	IQDFGLATEK	0.11	0.11	0.08	0.04	0.06	0.04	0.05	0.03	0.03	0.02	0.02	0.02	0.04	0.04	0.01	0.00	0.02	0.02
KD	563	UDAR	0.06	0.02	0.06	0.03	0.07	0.09	0.07	0.07	0.07	0.07	0.07	0.08	0.03	0.03	0.01	0.07	0.07	0.07
	573	WHGDVAVK	0.63	0.32	0.38	0.35	0.68	0.81	0.73	0.38	0.42	0.47	0.84	0.67	0.59	0.22	0.25	0.38	0.21	
	573	WHGDVAVK	0.64	0.33	0.39	0.36	0.69	0.82	0.74	0.39	0.42	0.47	0.85	0.68	0.60	0.23	0.26	0.44	0.24	
	573	WHGDVAVK	0.65	0.34	0.40	0.37	0.70	0.83	0.75	0.40	0.43	0.48	0.86	0.69	0.61	0.24	0.27	0.45	0.25	
	573	WHGDVAVK	0.66	0.35	0.41	0.38	0.71	0.84	0.76	0.41	0.44	0.49	0.87	0.70	0.62	0.25	0.28	0.46	0.26	
	573	WHGDVAVK	0.67	0.36	0.42	0.39	0.72	0.85	0.77	0.42	0.45	0.50	0.88	0.71	0.63	0.26	0.29	0.47	0.27	
	573	WHGDVAVK	0.68	0.37	0.43	0.40	0.73	0.86	0.78	0.43	0.46	0.51	0.89	0.72	0.64	0.27	0.30	0.48	0.28	
	573	WHGDVAVK	0.69	0.38	0.44	0.41	0.74	0.87	0.79	0.44	0.47	0.52	0.90	0.73	0.65	0.28	0.31	0.49	0.29	
	573	WHGDVAVK	0.70	0.39	0.45	0.42	0.75	0.88	0.80	0.45	0.48	0.53	0.91	0.74	0.66	0.29	0.32	0.50	0.30	
	573	WHGDVAVK	0.71	0.40	0.46	0.43	0.76	0.89	0.81	0.46	0.49	0.54	0.92	0.75	0.67	0.30	0.33	0.51	0.31	
	573	WHGDVAVK	0.72	0.41	0.47	0.44	0.77	0.90	0.82	0.47	0.50	0.55	0.93	0.76	0.68	0.31	0.34	0.52	0.32	
BRAF	339	SHPRDQEGDQEHR	1.79	1.62	2.03	2.63	2.52	1.76	1.02	2.32	3.32	2.02	1.15	0.83	3.44	1.90	1.49	2.65	1.36	
	339	SHPRDQEGDQEHR	1.81	1.64	2.05	2.65	2.53	1.78	1.03	2.33	3.33	2.03	1.16	0.84	3.45	1.91	1.50	2.66	1.37	
	445	DSSDOWEPIGQQTGQR	13.39	9.78	8.88	9.42	14.18	15.74	13.19	8.45	10.14	8.65	13.95	12.42	9.43	7.53	7.68	10.69	11.72	
	445	DSSDOWEPIGQQTGQR	14.01	9.49	8.58	9.12	10.59	12.09	13.09	8.53	10.25	9.59	13.52	12.04	9.44	7.50	7.65	10.67	11.67	
	445	DSSDOWEPIGQQTGQR	14.05	9.52	8.62	9.28	10.54	12.11	13.13	8.57	10.28	9.60	13.56	12.08	9.48	7.54	7.69	10.68	11.68	
	445	DSSDOWEPIGQQTGQR	14.07	9.54	8.64	9.30	10.56	12.13	13.15	8.59	10.30	9.62	13.58	12.10	9.50	7.56	7.71	10.70	11.70	
	445	DSSDOWEPIGQQTGQR	14.08	9.55	8.65	9.31	10.57	12.14	13.16	8.60	10.31	9.63	13.59	12.11	9.51	7.57</				

Region	Start Sequence	KSR1											
		Experiment 1				Experiment 2				Experiment 3			
		CNTRL	ADP	ATP	AMP-PNP	CNTRL	ADP	ATP	AMP-PNP	CNTRL	ADP	ATP	AMP-PNP
8	AAAMGEK	3.35	2.39	2.70	3.56	2.96	2.80	2.87	3.02	1.73	2.01	1.83	1.43
15	KEGGGGDAAEAGGAGAAAASR	2.56	2.61	2.65	3.46	3.00	2.86	2.20	2.64	1.94	1.98	1.88	1.61
16a	VTGLGGEEHKEDSSWSSLNDAR	0.71	0.56	0.33	1.09	0.82	0.57	0.24	0.52	0.62	0.65	0.45	0.61
177	EDSSWSSLNDAR	0.05	0.06	0.04	0.06	0.06	0.06	0.04	0.06	0.05	0.05	0.04	0.04
262	ALHSFITPPPTPQLR	4.17	6.90	4.44	5.01	8.44	5.64	3.06	3.78	8.63	5.99	4.99	4.93
318	IDDVSMR	0.28	0.28	0.25	0.36	0.36	0.33	0.14	0.38	0.16	0.23	0.17	0.15
401	RTEVPSDINNPVDR	4.63	4.66	4.73	5.27	5.03	5.21	3.96	4.91	4.40	4.31	3.96	3.72
416	AAEPHFGLTPK	3.55	0.00	3.23	4.70	3.66	3.50	2.89	3.37	2.56	2.69	2.64	2.25
549	ADVLEAHEAEAEPEAGKSEADEDEDEVDDLPSSR	0.47	0.58	0.41	0.64	0.55	0.43	0.42	0.63	0.37	0.38	0.34	0.30
567	SEAEDEDEDEVDDLPSSR	0.61	0.53	0.44	0.59	0.61	0.57	0.57	0.62	0.44	0.49	0.53	0.46
638	LLEMDGHNQDHDK	0.28	0.27	0.27	0.32	0.31	0.34	0.20	0.24	0.27	0.24	0.20	0.17
701	TSLDNK	0.20	0.18	0.16	0.28	0.20	0.21	0.15	0.20	0.11	0.14	0.10	0.07
KD	710 QIAQEIK	0.54	0.43	0.45	0.42	0.51	0.53	0.35	0.44	0.43	0.42	0.38	0.31
718	GMGYLHAK	1.39	1.25	0.80	0.84	1.59	1.20	0.68	1.05	1.23	1.23	0.82	0.88
840	VLTSVSLGK	0.02	0.02	0.02	0.00	0.03	0.02	0.02	0.04	0.02	0.01	0.02	0.01
894	SAEINSSK	1.02	0.90	0.92	1.30	1.06	1.14	0.73	1.06	0.71	0.73	0.70	0.53
909	FGLGVLESSNPK	0.35	0.32	0.31	0.46	0.41	0.42	0.26	0.35	0.25	0.26	0.22	0.22

D

Region	Start Sequence	B-Raf ^{WT} No Mg ²⁺											
		Experiment 1				Experiment 2				Experiment 3			
		Control 4h	ADP 4h	ATP 4h	AMP-PNP 4h	Control 4h	ADP 4h	ATP 4h	AMP-PNP 4h	Control 4h	ADP 4h	ATP 4h	AMP-PNP 4h
RBD	151 SPOKPV	0.09	0.11	0.07	0.04	0.22	0.33	0.21	0.12	0.79	0.96	0.41	0.34
	159 VFLPNK	0.02	0.03	0.02	0.01	0.04	0.05	0.03	0.02	0.84	0.75	0.41	0.35
	167 TVVPAR	0.04	0.09	0.07	0.04	0.15	0.14	0.06	0.13	0.51	0.62	0.35	0.35
	179 DSLKK	0.01	0.01	0.01	0.00	0.07	0.06	0.05	0.01	0.29	0.32	0.15	0.09
	184 ALMMR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.38	0.53	0.55
KD	339 SIPPQPFRPADEDHR	0.10	0.12	0.06	0.03	0.13	0.22	0.11	0.06	3.03	2.18	1.21	1.20
	355 NOFGQR	0.06	0.07	0.07	0.05	0.11	0.13	0.10	0.06	2.49	2.01	1.21	1.12
	390 GDGGSTTGLSATTPSPASLPGSLTNVK	0.01	0.01	0.01	0.00	0.01	0.01	0.02	0.01	0.09	0.11	0.05	0.05
	427 KSSSSSEDR	0.03	0.05	0.04	0.03	0.04	0.15	0.07	0.04	1.07	1.15	0.70	0.72
	445 DSSDDWEPDGQITVGQR	4.08	5.61	3.86	2.51	6.47	8.36	5.71	3.57	22.92	18.75	18.41	18.25
	463 IGSGSFTVYK	0.08	0.12	0.08	0.05	0.18	0.21	0.15	0.09	0.78	0.74	0.45	0.44
	474 GKVHGDVAVK	0.24	0.24	0.22	0.13	0.44	0.43	0.37	0.21	1.53	1.54	1.02	1.05
	500 NEVGVL	0.00	0.00	0.00	0.00	0.05	0.04	0.06	0.08	0.08	0.08	0.03	0.03
	553 LIDIR	0.02	0.01	0.01	0.00	0.03	0.04	0.03	0.01	0.20	0.16	0.07	0.02
	559 QTAAQGMDYLHAK	3.92	6.09	4.95	3.33	10.30	10.27	10.09	5.52	1.57	1.43	0.94	1.03
RBD	592 IGDFGLATVK	0.07	0.00	0.01	0.01	0.02	0.04	0.04	0.02	0.27	0.25	0.18	0.18
	663 DQIFMVGVR	1.87	0.08	0.04	0.02	0.19	0.20	0.13	0.03	0.01	0.01	0.00	ND
	672 GYLSPLDSLK	0.02	0.02	0.01	0.01	0.04	0.03	0.03	0.01	0.29	0.30	0.12	0.13
	727 SASEPSLNR	1.10	1.10	0.99	0.54	2.23	2.10	1.86	1.00	7.25	6.42	4.46	4.42
	753 TPIQAGGYGAFPVH	0.19	0.46	0.25	0.23	0.53	0.73	0.55	0.36	3.44	3.18	2.07	2.08
	With Mg ²⁺												
	RBD	Experiment 1				Experiment 2				Experiment 3			
		Control 4h	ADP 4h	ATP 4h	AMP-PNP 4h	Control 4h	ADP 4h	ATP 4h	AMP-PNP 4h	Control 4h	ADP 4h	ATP 4h	AMP-PNP 4h
		0.18	0.08	0.15	0.29	0.52	0.69	0.46	0.39	0.42	0.50	0.39	0.39
		0.01	0.01	0.01	0.01	0.14	0.12	0.11	0.07	0.05	0.18	0.09	0.14
		0.03	0.05	0.09	0.01	0.31	0.48	0.34	0.24	0.23	0.17	0.22	0.28
	KD	0.05	0.03	0.04	0.03	0.24	0.30	0.27	0.18	0.13	0.12	0.17	0.15
		0.00	0.02	0.03	0.03	0.90	0.20	0.45	1.17	0.55	0.91	0.62	0.62
		0.59	0.81	0.65	0.88	2.33	3.65	2.18	1.19	2.55	2.89	2.10	2.52
		0.13	0.18	0.23	0.18	1.02	1.35	1.21	0.88	1.04	0.95	1.11	0.97
		0.03	0.04	0.04	0.04	0.55	0.51	0.31	0.54	0.22	0.17	0.36	0.42
KD	427 KSSSSSEDR	1.71	1.72	1.70	2.20	2.95	5.27	5.18	3.41	4.89	6.72	4.31	5.36
	445 DSSDDWEPDGQITVGQR	3.88	4.55	4.30	4.22	19.32	22.10	18.03	13.21	18.48	18.93	16.22	16.09
	463 IGSGSFTVYK	0.05	0.07	0.06	0.06	0.42	0.48	0.25	0.30	0.32	0.30	0.25	0.29
	474 GKVHGDVAVK	0.11	0.17	0.18	0.16	1.09	1.30	0.89	0.95	0.88	0.77	0.92	0.79
	500 NEVGVL	0.03	0.08	0.09	0.06	0.06	0.05	0.03	0.04	0.03	0.02	0.04	0.03
	553 LIDIR	0.00	0.01	0.01	0.01	0.01	0.09	0.05	0.01	0.01	0.01	0.01	0.03
	559 QTAAQGMDYLHAK	1.87	3.75	3.70	4.03	0.59	0.75	0.47	0.41	0.42	0.36	0.45	0.41
	592 IGDFGLATVK	0.00	0.01	0.01	0.01	0.12	0.12	0.07	0.08	0.06	0.05	0.08	0.06
	663 DQIFMVGVR	0.01	0.02	0.04	0.04	0.02	0.01	0.01	0.01	0.00	0.00	0.01	0.01
	672 GYLSPLDSLK	0.00	0.01	0.01	0.01	0.13	0.14	0.10	0.08	0.07	0.06	0.08	0.07
RBD	727 SASEPSLNR	0.61	0.82	0.96	0.84	3.44	3.92	3.37	2.92	2.87	2.75	3.27	2.97
	753 TPIQAGGYGAFPVH	0.17	0.25	0.25	0.23	1.46	1.54	1.20	0.99	0.97	0.76	1.26	1.07

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