Supplementary Material for:

Docking Interactions of Hematopoietic Tyrosine Phosphatase (HePTP) with the MAP Kinases ERK2 and p38α

Andrea Piserchio, Dana M Francis, Dorothy Koveal, Kevin N. Dalby, Rebecca Page, Wolfgang Peti, and Ranajeet Ghose.

Materials and Methods

Uniformly $^{2}H,^{13}C,^{15}N$-labeled resting ERK2[1] (called ERK2 from hereon forward), unlabeled full-length HePTP[2] and the KIMKIS peptide (encoding HePTP residues 15-56 and containing a C42S mutation)[3] were expressed and purified as described before. Unlabeled KIM_{15-31} peptide (^{15}VRLQERRGSNVALMLDV^{31}) was purchased from BioSynthesis and used following published protocols[3]. All NMR samples were prepared in NMR buffer (50 mM HEPES, 150 mM NaCl, 5 mM DTT, at pH 6.8 containing 10% D_2O). For the NMR-based titration experiments, the KIM_{15-31} peptide was dissolved from a concentrated stock into the NMR buffer and added incrementally to an NMR sample containing 130 μM ERK2 (final peptide concentrations: 0, 50, 100 150, 200, 250, 350, 1000 μM). A 2D ^{1}H, ^{15}N-TROSY was collected for each titration point, and 3D TROSY-based HNCO[4] experiments were collected for the first and the last two titration points. All experiments were acquired at 25 °C on a Varian Inova 600 MHz spectrometer equipped with a triple resonance cryogenic probe capable of apply pulsed field gradients on the z-axis.

The complex between ERK2 and full-length HePTP was prepared by mixing equimolar amounts of the two purified proteins and additional purification of their 1:1 complex was carried out using size exclusion chromatography (Superdex 75 16/60, GE HealthCare Biosciences; pre-equilibrated in NMR buffer). The elution peak corresponding to the 1:1 complex was concentrated to a final protein concentration of ~250 μM. 2D ^{1}H, ^{15}N-TROSY and 3D TROSY-based HNCO experiments were collected at 25 °C on a Bruker Avance 800 MHz spectrometer equipped with a triple resonance

cryogenic probe capable of applying pulsed field gradients on the z-axis. All data were processed using NMRpipe[5] and analyzed using Sparky.

Chemical shifts perturbations were calculated using the formula:

$$\Delta\delta = \sqrt{(\Delta\delta_{\cdot})^2 + (0.1\Delta\delta_{\cdot})^2} \quad (1)$$

Thresholds to identify statistically relevant chemical shift perturbations in ERK2 were determined as described by Schumann *et. al.*[6] and were found to be 0.041 ppm for KIM_{15-31} and 0.052 ppm for full-length HePTP. Perturbations for p38α have been published previously[3]. The amino-acid sequences of the two kinases were aligned using ClustalW2[7] and the results were used for comparison of the spectral perturbations for corresponding residues in ERK2 and p38α.

For the Isothermal Titration Calorimetry (ITC) measurements, HePTP and ERK2 were equilibrated in the identical buffer (10 mM HEPES, 150 mM NaCl, 0.1 mM EDTA, 0.5 mM TCEP at pH 7.5) and each protein was purified using size exclusion chromatography (Superdex 75 26/60; GE HealthCare Biosciences) immediately prior to the experiment. The KIM_{15-31} peptide was solubilized in the identical buffer and the KIMKIS peptide was dialyzed into the identical buffer at 4 °C for 18 hours before the experiment. ITC experiments were performed at 25 °C using a VP-ITC microcalorimeter (Microcal Inc.). Titrant (10 μL per injection) was injected into the sample cell over a period of 20 seconds with a 250 second interval between injections to allow for complete equilibration and baseline recovery. 28 injections (28 titration points) were delivered during each experiment, and the solution in the sample cell was stirred at 307 rpm to ensure rapid mixing. To determine the thermodynamic parameters (ΔH, ΔS, ΔG) and the binding constant (K_d) for the ERK2:HePTP interaction, HePTP was titrated into ERK2.

To determine the corresponding parameters for the ERK2:KIMKIS and the ERK2:KIM_{15-31} interactions, ERK2 was titrated into the KIMKIS and KIM_{15-31} peptides, respectively. For all titrations the concentrations were varied slightly to ensure independence of the calculated K_d from the protein/peptide concentrations. Data were analyzed using a one-site binding model assuming a 1:1 binding stoichiometry using the Origin 7.0 (OriginLab) software. A nonlinear least-squares algorithm and the titrant and sample cell concentrations were used to fit the heat flow per injection to an equilibrium binding equation, providing values of the stoichiometry (n), change in enthalpy (ΔH), and binding constant (K_d). All data were collected in triplicate to confirm reproducibility.

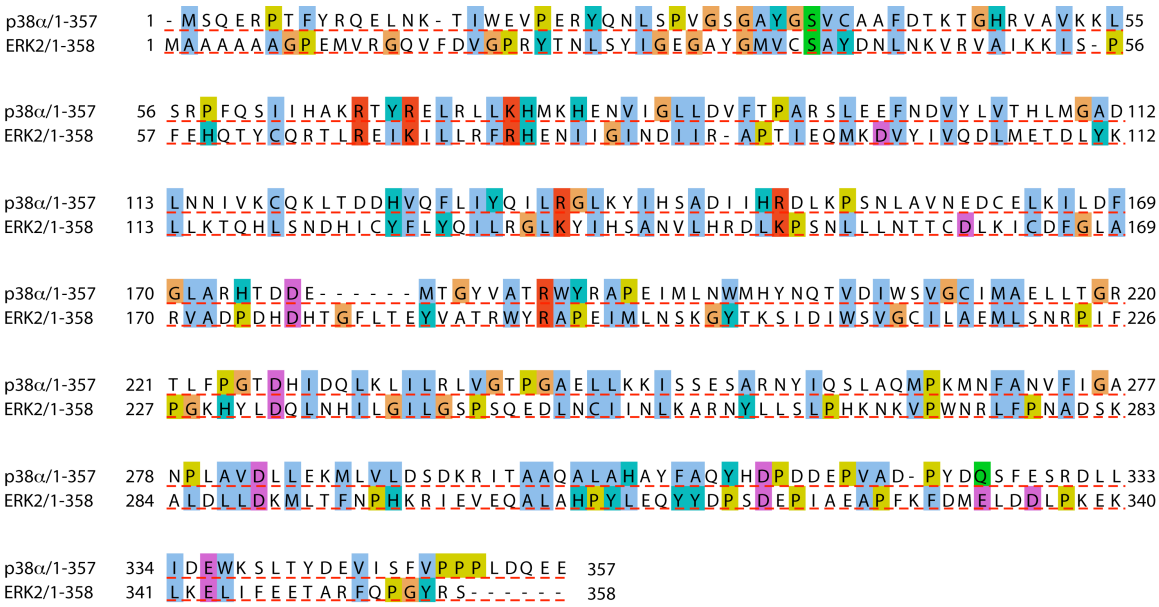

Figure S1. Alignment of the sequences of p38α (*Mus musculus,* NCBI: NP_036081.1) and ERK2 (*Rattus norvegicus,* NCBI: NP_446294.1). The two sequences are 42% identical. The alignment was used for the pairwise comparison of spectral perturbations induced by KIM_{15-31} and full-length HePTP on ERK2 and p38α. A ClustalX coloring scheme is used - red: R, K; magenta: D, E; blue: W, M, I, L, V, A, F, C; Green: Y, H, N, Q, S, T; orange: G; yellow: P. P and G are colored whether conserved or not.

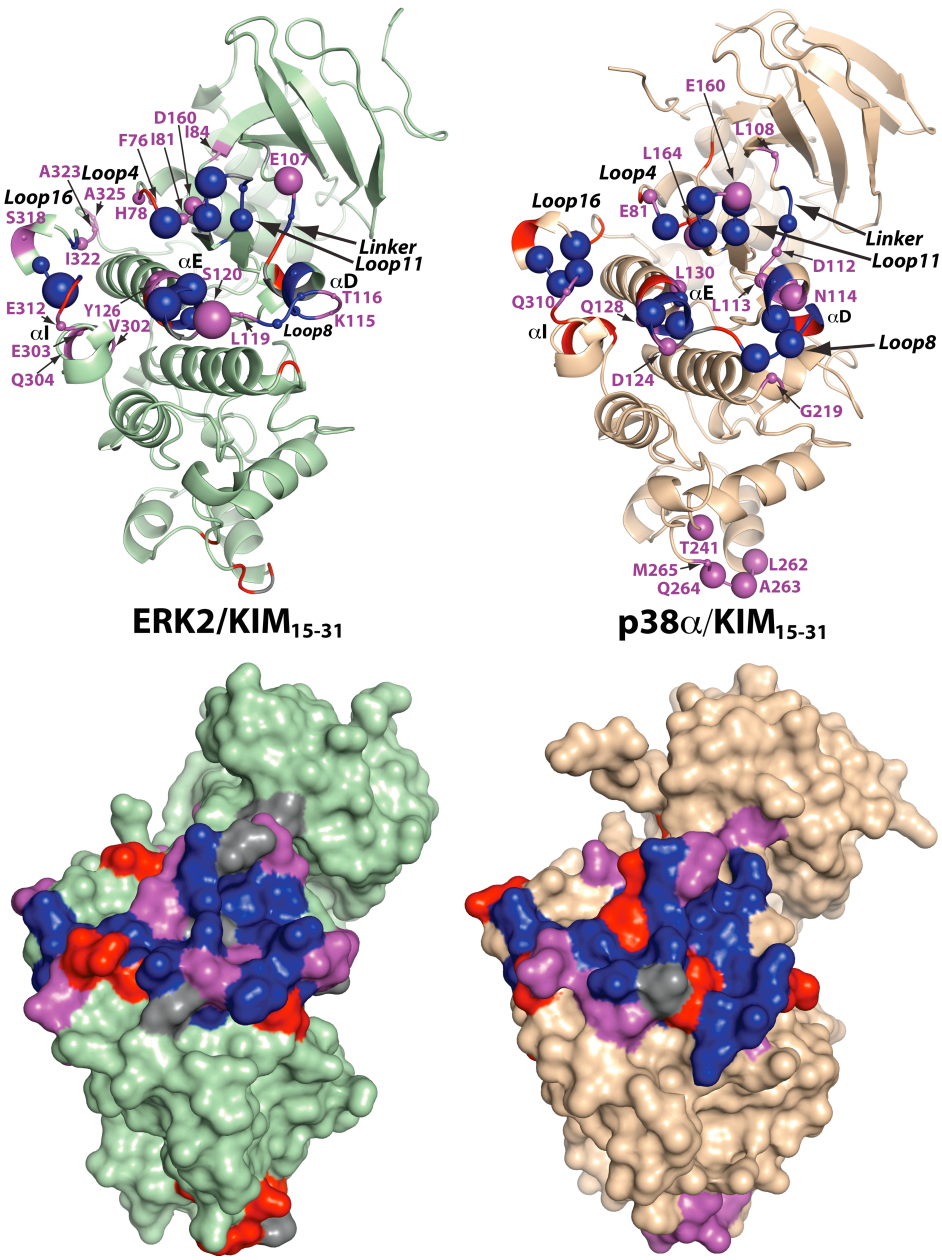

Figure S2. Comparison of the perturbations induced by KIM_{15-31} on ERK2 (left) and p38α (right) using ribbon (top) and surface (bottom) representations. The radii of the spheres on the top panel are proportional to the relative magnitude of the chemical shift perturbations. For visualization purposes an arbitrary value of 0.32 ppm was assigned to disappearing resonances, and the magnitude of the perturbation of the ERK2 residue S120 was rescaled from 0.79 to 0.32 ppm. The color-coding on the ERK2 plots are

defined as follows: ERK2 residues that were perturbed by KIM_{15-31} are colored blue if the corresponding residues on p38α were also perturbed, or magenta if the corresponding residues on p38α were not perturbed. The latter residues are labeled on the ribbon representation. ERK2 residues are colored red if they were not perturbed by KIM_{15-31} but the corresponding residues on p38α were perturbed. Finally, ERK2 residues were colored gray if they were unassigned and the corresponding residues on p38α were perturbed by KIM_{15-31}. Similar color-coding and labeling schemes were used for the p38α plots (right).

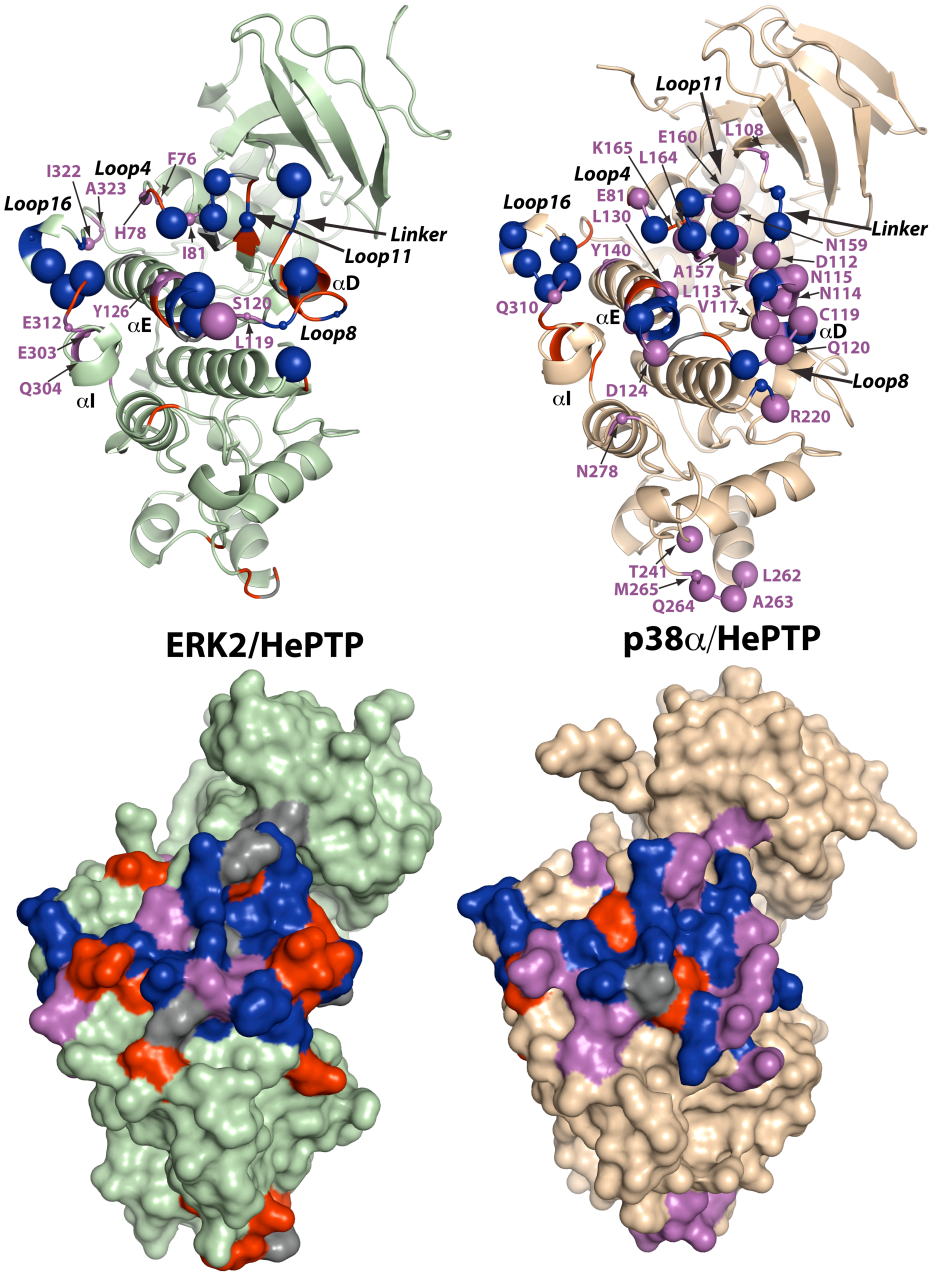

Figure S3. Comparison of the spectral perturbations induced by full-length HePTP on ERK2 (left) and p38α (right) using ribbon (top) and surface (bottom) representations. The radii of the spheres on the top panel are proportional to the magnitude of the chemical shift perturbations. For visualization purposes (as in **Figure S2**), an arbitrary value of 0.25 ppm was assigned to the disappearing resonances. A color scheme identical to that described for **Figure S2** is used.

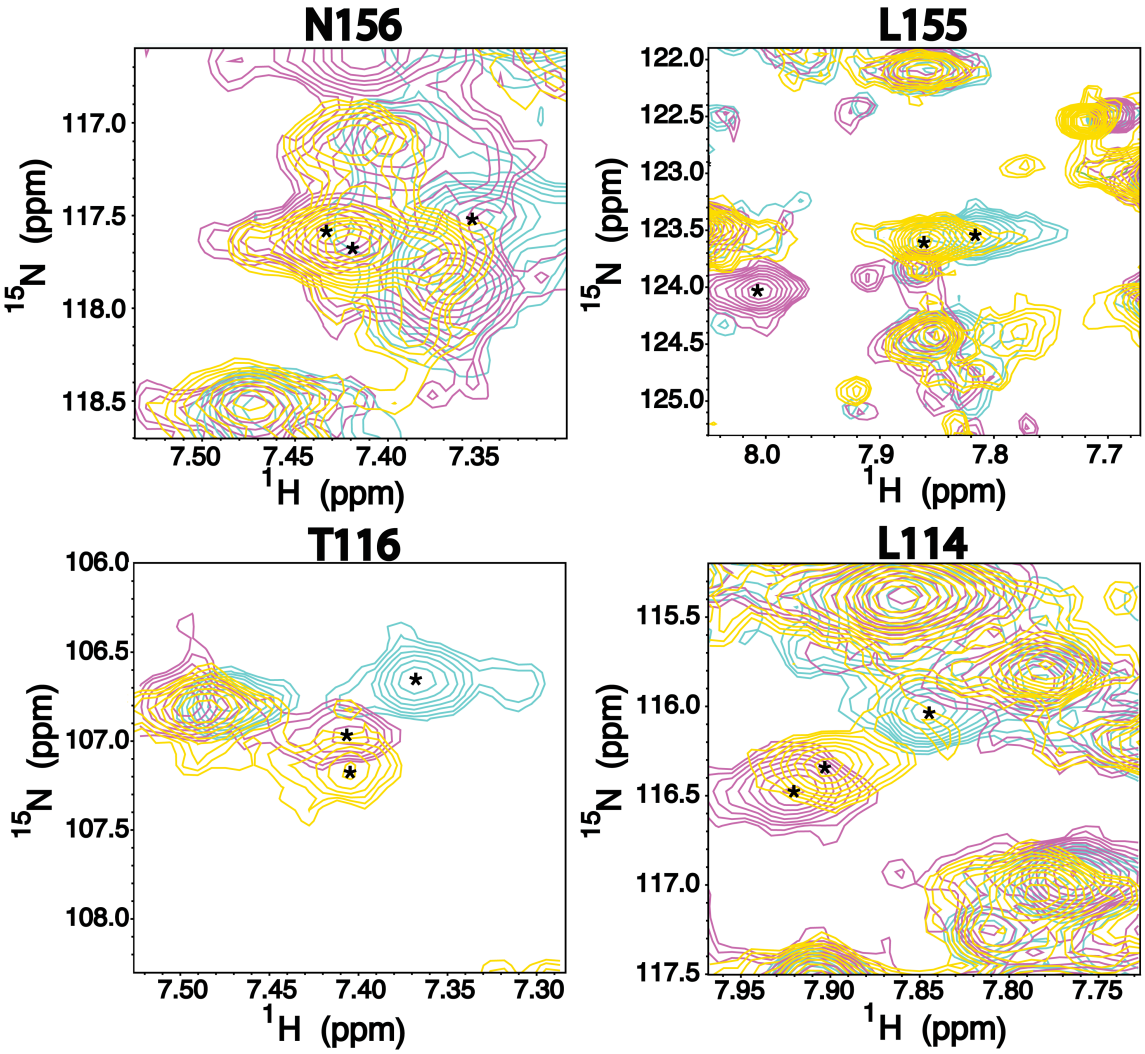

Figure S4. Comparison of $^{15}N,^{1}H$ TROSY resonances belonging to a subset of ERK2 residues for which the relative magnitude of the chemical shift changes is *lower* in the presence of full-length HePTP than in the presence of $KIM_{15\text{-}31}$. In each inset, resonances (positions indicated by the *) corresponding to free ERK2 (magenta), ERK2 bound to full-length HePTP (gold) and ERK2 bound to $KIM_{15\text{-}31}$ (moss) are shown.

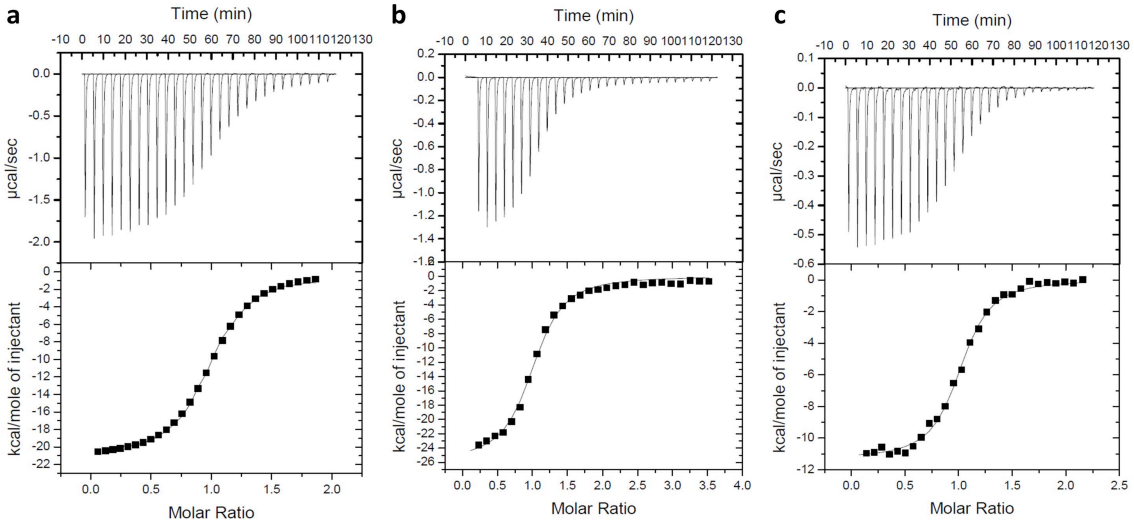

Figure S5. Raw ITC data (upper panels) and the corresponding derived binding isotherms plotted against the molar ratio of titrant fit using a one-site model (lower panels) for (**a**) ERK2 titrated with KIM_{15-31}, (**b**) ERK2 titrated with KIMKIS, and (**c**) ERK2 titrated with full-length HePTP.

Table S1. Overview of the chemical shift perturbations (CSP) induced by KIM_{15-31} on ERK2.

Residue Number	Amino Acid	Perturbation (ppm)
76	**F**	**0.077**
78	H	0.120
79	E	0.035
80	N	0.292
81	I	0.112
82	I	0.049
84	I	0.044
105	L	Unassigned
107	E	0.256
108	T	0.059
109	D	0.027
110	L	Unassigned
111	Y	Unassigned
113	L	0.094
114	L	0.090
115	K	0.043
116	T	0.051
117	Q	0.069
118	H	0.086
119	L	0.052
120	S	0.789
121	N	Unassigned
122	D	0.295
123	H	0.276
124	I	0.198
125	C	0.030
126	Y	0.097
127	F	Unassigned
143	V	0.052
151	S	0.019
155	L	0.205
155	L	0.205
156	N	0.080
157	T	Unassigned
158	T	0.275
159	C	0.248
160	D	0.169
161	L	Unassigned
164	C	0.053
222	N	0.035
244	S	0.017
265	L	0.002
266	P	Unassigned
267	H	0.013
268	K	0.012
302	V	0.058
303	E	0.096
304	Q	0.072

312	E	0.087
313	Q	0.019
314	Y	0.046
315	Y	Vanished
316	D	0.157
318	S	0.066
320	E	0.065
322	I	0.126
323	A	0.060
325	A	0.052

The color-coding scheme as described for **Figures S1** and **S2** has been used. Thus, values above the threshold are shaded blue (when the corresponding residue also perturbed in p38α) and magenta (residue perturbed only in ERK2). Residues for which the perturbations lie below the threshold in ERK2 but the corresponding positions are perturbed in p38α, are shaded red. Gray shading represents residues unassigned in ERK2 but the corresponding position is perturbed in p38α.

Table S2: Overview of the CSP induced by full-length HePTP on ERK2.

Residue Number	Amino Acid	Perturbation (ppm)
76	F	0.076
78	H	0.126
79	E	0.041
80	N	0.292
81	I	0.124
82	I	Unassigned
105	L	Unassigned
107	E	Vanished
108	T	0.057
109	D	0.027
110	L	Unassigned
111	Y	Unassigned
112	K	0.003
113	L	Vanished
114	L	0.024
115	K	0.060
116	T	0.019
117	Q	0.021
118	H	0.075
119	L	0.068
120	S	Vanished
121	N	Unassigned
122	D	0.078
123	H	Vanished
124	I	Vanished
125	C	0.033
126	Y	0.114
127	F	Unassigned
137	Y	Unassigned
151	S	0.010
154	L	0.008
155	L	0.151
156	N	0.011
157	T	Unassigned
158	T	0.233
159	C	0.243
161	L	Unassigned
162	K	Unassigned
163	I	Unassigned
222	N	Vanished
223	R	0.023
244	S	0.006
265	L	0.012
266	P	Unassigned
267	H	0.017
268	K	0.013
281	D	0.028
301	E	0.056
303	E	0.094
304	Q	0.074
312	E	0.073
313	Q	0.020

314	Y	Vanished
315	Y	Vanished
316	D	Vanished
318	S	0.069
320	E	0.071
322	I	0.122
323	A	0.055

The color-coding scheme is the same as in **Figures S1** and **S2**, and described at length for **Table S1** (above).

Table S3. Thermodynamic parameters and dissociation constants for the interactions of HePTP, KIMKIS and KIM_{15-31} with ERK2 and p38α derived from ITC measurements at 25 °C.

Complex	K_d (nM)		ΔH (kcal·mol^{-1})		TΔS (kcal·mol^{-1})		ΔG (kcal·mol^{-1})	
	ERK2	**p38α**	**ERK2**	**p38α**	**ERK2**	**p38α**	**ERK2**	**p38α**
HePTP	371 ± 40	2570 ± 650	-11.5 ± 0.2	-20.5 ± 2.3	-2.7 ± 0.3	-12.9 ± 2.2	-8.8 ± 0.1	-7.6 ± 0.1
KIMKIS	679 ± 122	778 ± 28	-24.4 ± 1.7	-24.9 ± 1.0	-15.9 ± 1.7	-16.6 ± 1.0	-8.4 ± 0.1	-8.3 ± 0.0
KIM_{15-31}	1277 ± 110	5150 ± 1290	-20.7 ± 2.5	-19.6 ± 5.5	-12.7 ± 2.5	-12.4 ± 5.6	-8.0 ± 0.1	-7.2 ± 0.1

Data represent mean ± one standard deviation derived from measurements in triplicate. Data for p38α from Francis et. al.[3]

References

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