SUPPLEMENTAL INFORMATION FOR:

The differential regulation of p38α by the neuronal KIM-PTPs, a detailed molecular study

Dana M. Francis, Ganesan Senthil Kumar, Dorothy Koveal, Antoni Tortajada, Rebecca Page and Wolfgang Peti

Inventory of Supplemental Information:

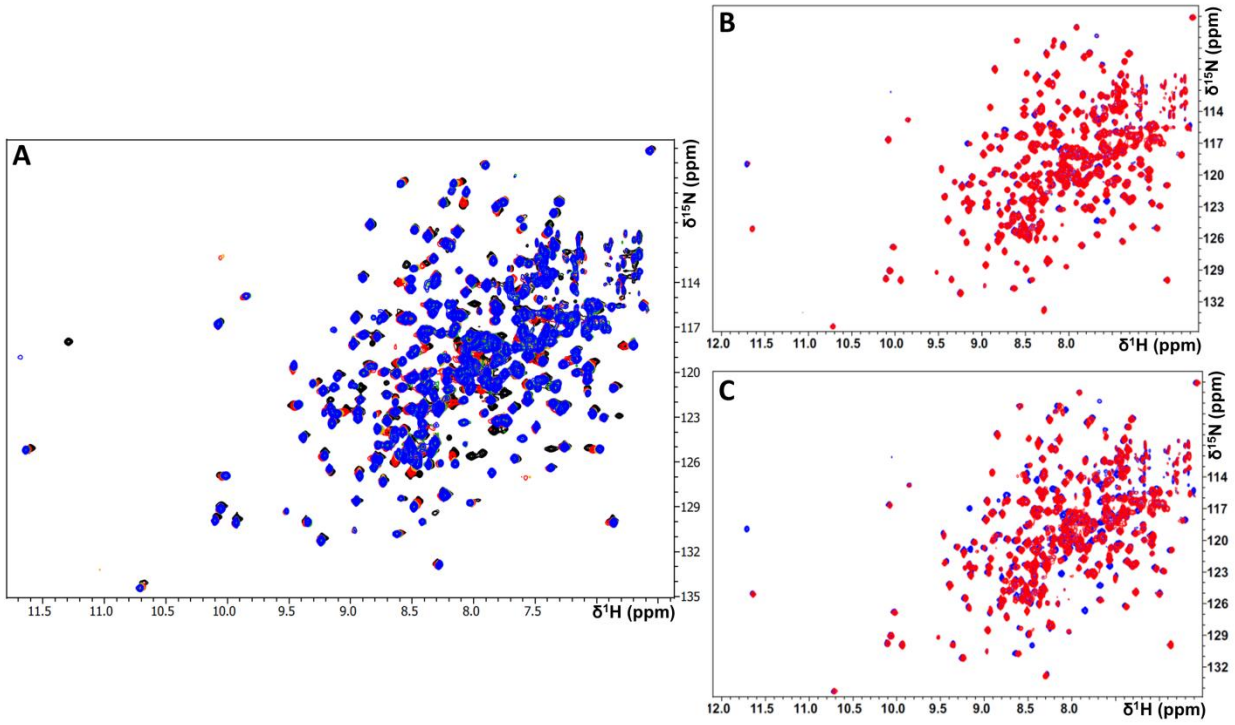


Figure S1: Comparison of KIM-PTP$_{KIM}$ binding to [^{2}H,^{15}N]-p38α, *related to Figure 1*
(**A**) Overlay 2D [^{1}H,^{15}N] TROSY spectra of [^{2}H,^{15}N]-p38α (black), p38α:STEP$_{KIM}$ 1:1 (red), p38α:STEP$_{KIM}$ 1:3 (orange), p38α:STEP$_{KIM}$ 1:5 (green), p38α:STEP$_{KIM}$ 1:8 (blue).
(**B**) 2D [^{1}H,^{15}N] TROSY spectrum of [^{2}H,^{15}N]-p38α:PTPSL$_{KIM}$ (blue; 1:8 molar ratio p38α:PTPSL$_{KIM}$) overlaid with the 2D [^{1}H,^{15}N]-TROSY spectrum of [^{2}H,^{15}N]-p38α:STEP$_{KIM}$ (red; 1:8 molar ratio p38α:STEP$_{KIM}$).
(**C**) 2D [^{1}H,^{15}N] TROSY spectrum of [^{2}H,^{15}N]-p38α:PTPSL$_{KIM}$ (blue; 1:8 molar ratio p38α:PTPSL$_{KIM}$) overlaid with the 2D [^{1}H,^{15}N]-TROSY spectrum of [^{2}H,^{15}N]-p38α:HePTP$_{KIM}$ (red; 1:8 molar ratio p38α:HePTP$_{KIM}$).

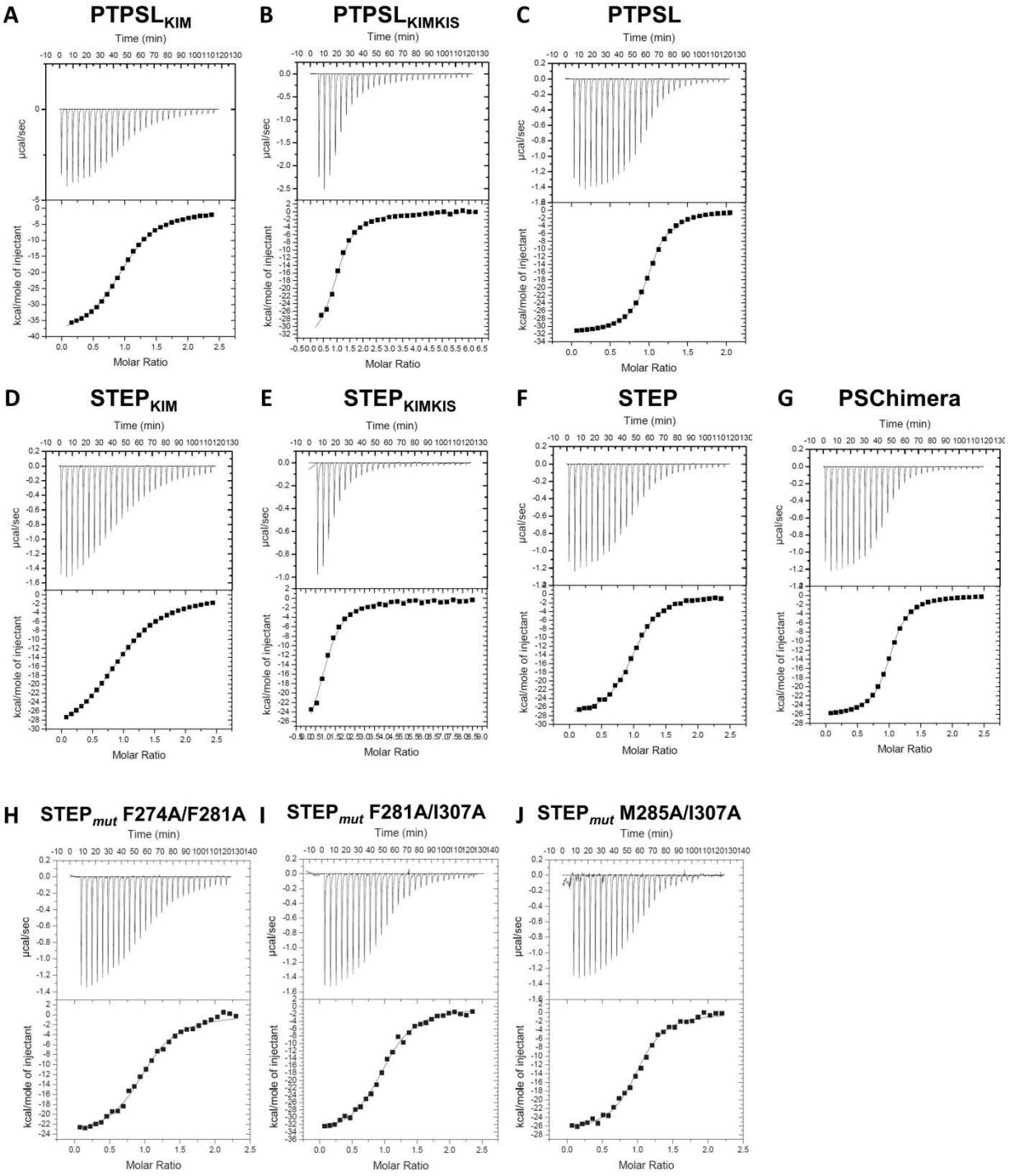
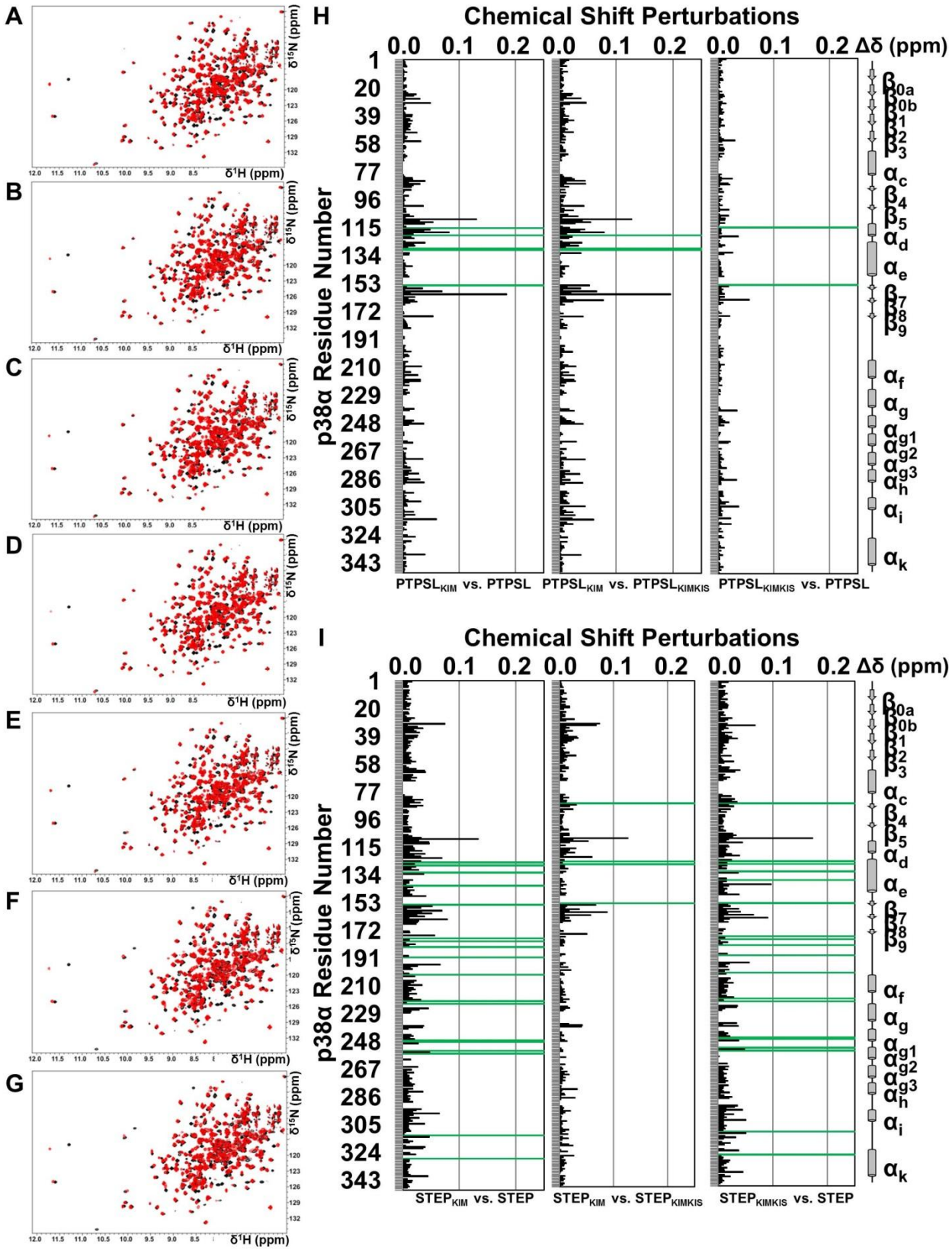


Figure S2: Raw isothermal titration calorimetry data, *related to Table 1*
Raw isothermal titration calorimetry data (upper panels) and derived binding isotherm plotted vs. the molar ratio of titrant fit using a one-site model (lower panels) for p38α with (**A**) PTPSL$_{KIM}$, (**B**) PTPSL$_{KIMKIS}$, (**C**) PTPSL, (**D**) STEP$_{KIM}$, (**E**) STEP$_{KIMKIS}$, (**F**) STEP, (**G**) PSChimera, (**H**) STEP$_{mut}$ F274A/F281A, (**I**) STEP$_{mut}$ F281A/I307A and (**J**) STEP$_{mut}$ M285A/I307A.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C
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δ¹H (ppm)
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Chemical Shift Perturbations
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p38α Residue Number
PTPSL_KIM vs. PTPSL
PTPSL_KIM vs. PTPSL_KIMKIS
PTPSL_KIMKIS vs. PTPSL
I
Chemical Shift Perturbations
STEP_KIM vs. STEP
STEP_KIM vs. STEP_KIMKIS
STEP_KIMKIS vs. STEP

Figure S3: Spectra and comparison of interactions between [^{2}H,^{15}N]-p38α and various KIM-PTP domains, *related to Figure 2*

2D [^{1}H,^{15}N] TROSY spectra of [^{2}H,^{15}N]-p38α (black) overlaid with those obtained for [^{2}H,^{15}N]-p38α in complex with the following constructs (red): (**A**) $PTPSL_{KIM}$ (1:8 molar ratio), (**B**) $PTPSL_{KIMKIS}$ (1:3 molar ratio), (**C**) PTPSL (1:1 ratio; complex purified by SEC), (**D**) $STEP_{KIM}$ (1:8 molar ratio), (**E**) $STEP_{KIMKIS}$ (1:8 molar ratio), (**F**) STEP (1:1 ratio; complex purified by SEC), and (**G**) PSChimera (1:1 ratio; complex purified by SEC). (**H**) Histograms which show the combined ^{1}H/^{15}N CSP vs. p38α residue for each PTPSL construct with [^{2}H,^{15}N]-p38α versus each other. (**I**) Histograms which show the combined ^{1}H/^{15}N CSP vs. p38α residue for each STEP construct with [^{2}H,^{15}N]-p38α versus each other.

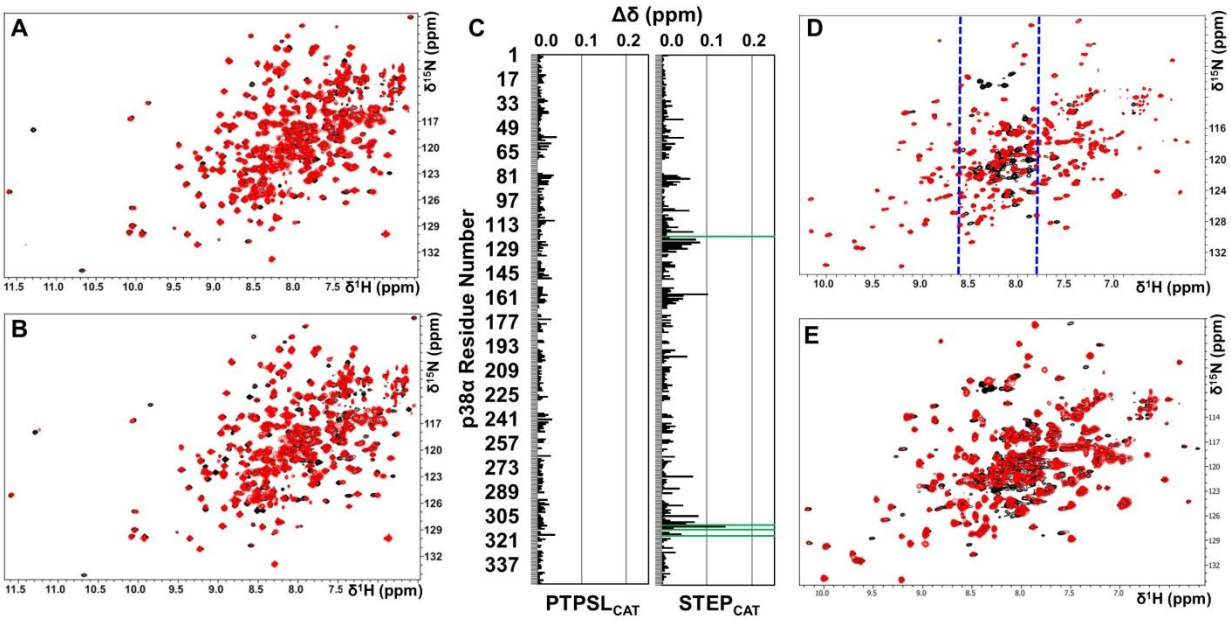

Figure S4: Characterization of the interaction between PTPSL$_{CAT}$ and STEP$_{CAT}$ with p38α, *related to Figure 4*

(**A**) 2D [^{1}H,^{15}N] TROSY spectrum of [^{2}H,^{15}N]-p38α (black) overlaid with the 2D [^{1}H,^{15}N]-TROSY spectrum of [^{2}H,^{15}N]-p38α:PTPSL$_{CAT}$ (red; 1:2 molar ratio)

(**B**) 2D [^{1}H,^{15}N] TROSY spectrum of [^{2}H,^{15}N]-p38α (black) overlaid with the 2D [^{1}H,^{15}N]-TROSY spectrum of [^{2}H,^{15}N]-p38α:STEP$_{CAT}$ (red; 1:2 molar ratio)

(**C**) Histograms which show the combined ^{1}H/^{15}N CSP vs. p38α residue for p38α with PTPSL$_{CAT}$ (1:2 molar ratio) and p38α with STEP$_{CAT}$ (1:2 molar ratio).

(**D**) 2D [^{1}H,^{15}N] TROSY spectra of [^{2}H,^{15}N]-STEP (black) overlaid with the 2D [^{1}H,^{15}N]-TROSY spectra of [^{2}H,^{15}N]-STEP$_{CAT}$ (red). 30 additional peaks that cluster between 7.8-8.6 ppm are observed in the spectrum of STEP versus STEP$_{CAT}$.

(**E**) 2D [^{1}H,^{15}N] TROSY spectra of [^{2}H,^{15}N]-STEP (black) overlaid with the 2D [^{1}H,^{15}N]-TROSY spectra of the [^{2}H,^{15}N]-STEP:p38α complex (red).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Isothermal Titration Calorimetry

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 titrations to allow for complete equilibration and baseline recovery. 28 injections 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 binding constants (K) of the p38α: $PTPSL_{KIM}$, p38α: PTPSL, and p38α:STEP interactions, $PTPSL_{KIM}$, PTPSL, and STEP were titrated into the p38α. To determine the same parameters for the p38α:$STEP_{KIM}$, p38α:$PTPSL_{KIMKIS}$, p38α:$STEP_{KIMKIS}$, and p38α:PSChimera interaction, p38α was titrated into the protein. For all titrations the concentrations were slightly varied to ensure independence of the K_d from the protein/peptide concentrations. Data were analyzed with a one-site binding model assuming a binding stoichiometry of 1:1 using Origin 7.0 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). All data were repeated in triplicate.

SAXS data collection

All data was recorded at beamline X9 at the National Synchrotron Light Source (NSLS) using a Dectris pilatus 300k (p38α:PTPSL and active-state p38α:STEP; 3.4 m distance from the sample for SAXS) or a MarCCD 165 (resting-state p38α:STEP; 3.4 m distance from the sample for SAXS) and a Photonic Science CCD (0.47 m from the sample for WAXS) detector.

Purification of constitutively-active MKK6

$MKK6_{1-334}$ S207E/T211E was subcloned into a derivative of the pET28 vector that includes an N-terminal His_6-tag and a TEV protease recognition sequence and expressed in BL21 (DE3) RIL cells. His_6-$MKK6_{1-334}$ S207E/T211E was purified using immobilized metal affinity chromatography (IMAC). Following overnight cleavage with TEV protease in 50 mM Tris pH 8.0, 500 mM NaCl (4˚C), $MKK6_{1-334}$ S207E/T211E was further purified using a second IMAC column followed by SEC (Superdex 75 26/60). The purified protein was frozen at -80ºC until it was used in phosphorylation assays.

Phosphorylation of p38α

To generate dually phosphorylated p38α, a molar ratio of 1:40 of MKK6-EE:p38α was used for all phosphorylation assays. The reaction components were added to a 50 ml conical tube in the following order to achieve the reported final concentrations in a 30 ml volume: 20 mM HEPES pH 7.5, 0.5 mM EDTA, 2 mM DTT, 20 mM MgCl2, 0.05 µM MKK6-EE, 2 µM p38, 4 mM ATP. The mixture was mixed by pipetting up/down several times and was incubated at 27 °C for 5 min prior to adding ATP. After the addition of ATP, the mix was incubated in a water bath at 27 °C for 3 h. Following incubation, ATP was removed by filtering (Amicon Ultra-15, EMD Millipore) using buffer A (20 mM Tris pH 8.0, 2 mM DTT). After filtration to remove precipitant, the sample was purified by IEC

(Mono Q 5/50 GL; GE Healthcare). Dually phosphorylated p38α was eluted using a 0-375 mM NaCl gradient over 91.5 column volumes; the protein eluted at 228 mM NaCl and was concentrated to 7 mg/ml and frozen and stored until usage at -80°C. Correct phosphorylation of p38α was verified by ESI-MS.

Purification of the substrate-trapping mutant of STEP

The substrate-trapping mutant of STEP was generated based on previously published studies (Critton et al., 2008). T306D and C472S mutations were introduced into $STEP_{213-539}$ already subcloned into a derivative of the pET28 vector that includes an N-terminal His_6-tag and a TEV protease recognition sequence using the QuikChange Mutagenesis Kit (Agilent Technologies). All constructs were verified by sequencing (Beckman Coulter). $STEP_{213-539}$ T306D/C472S was expressed and purified using the protocol previously described in detail for $STEP_{CAT}$ (Francis et al., 2013).

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Critton, D.A., Tortajada, A., Stetson, G., Peti, W., and Page, R. (2008). Structural basis of substrate recognition by hematopoietic tyrosine phosphatase. Biochemistry *47*, 13336-13345.

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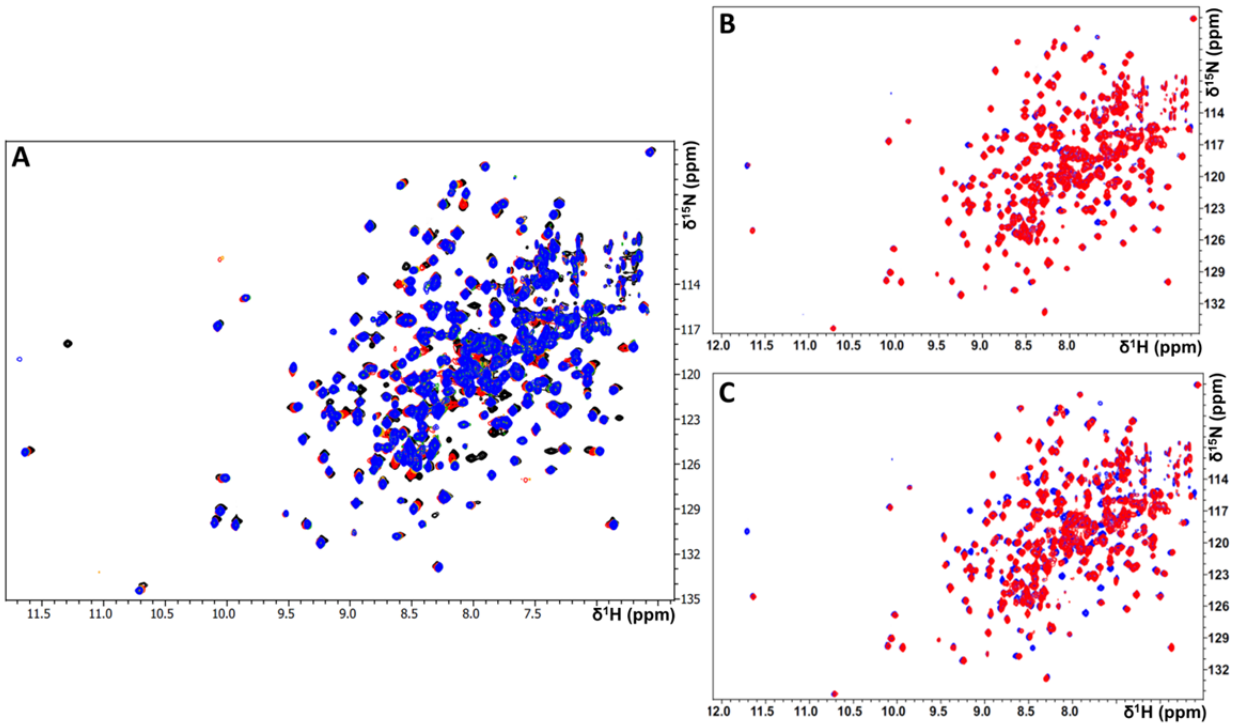

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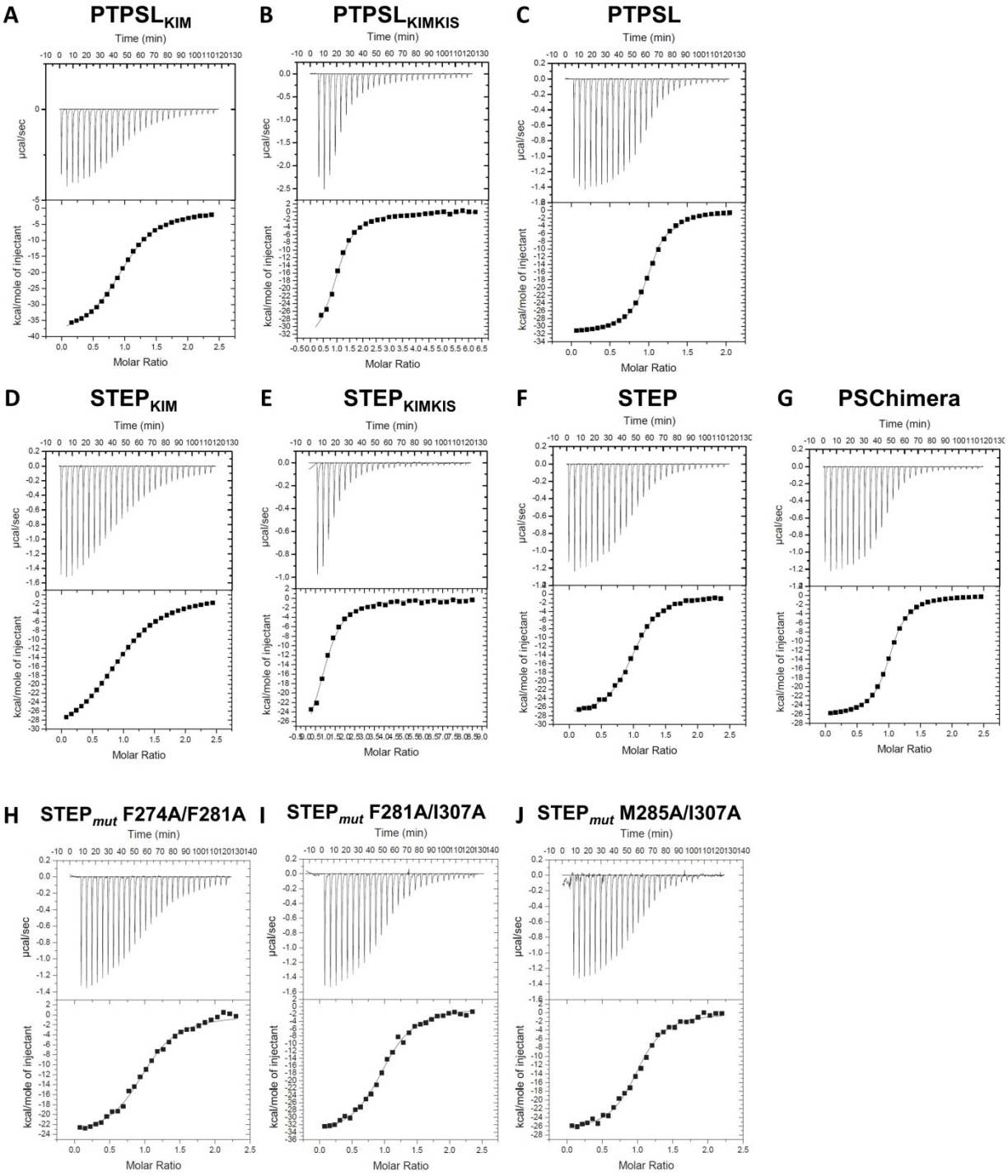

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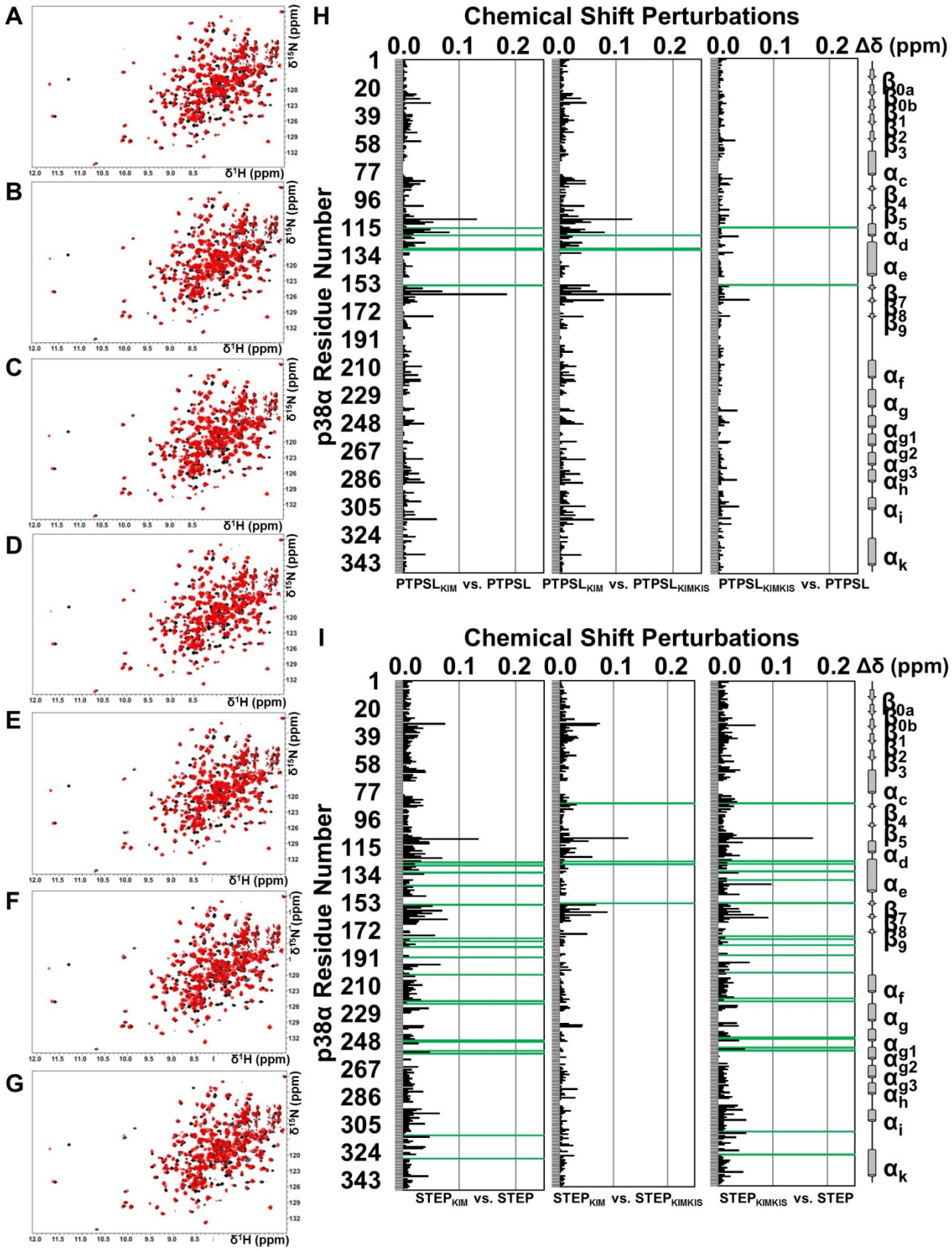
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δ¹⁵N (ppm)
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Chemical Shift Perturbations
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p38α Residue Number
PTPSL_KIM vs. PTPSL
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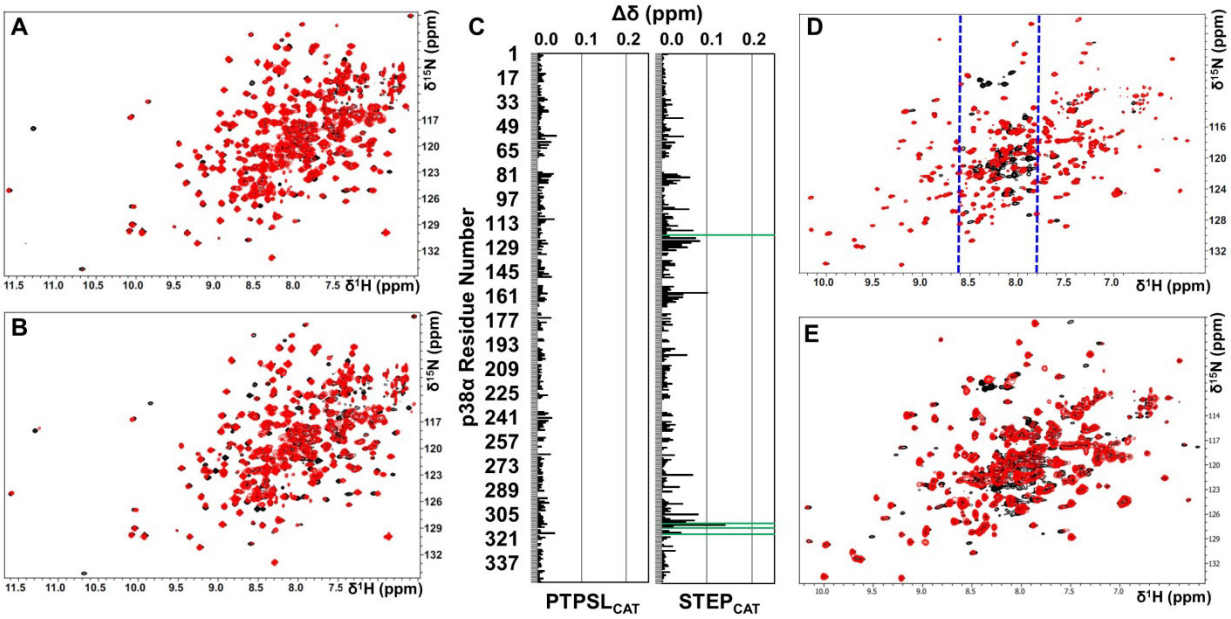

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