

Supplementary Materials for

A Distinct Interaction Mode Revealed by the Crystal Structure of the Kinase p38 α with the MAPK Binding Domain of the Phosphatase MKP5

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References

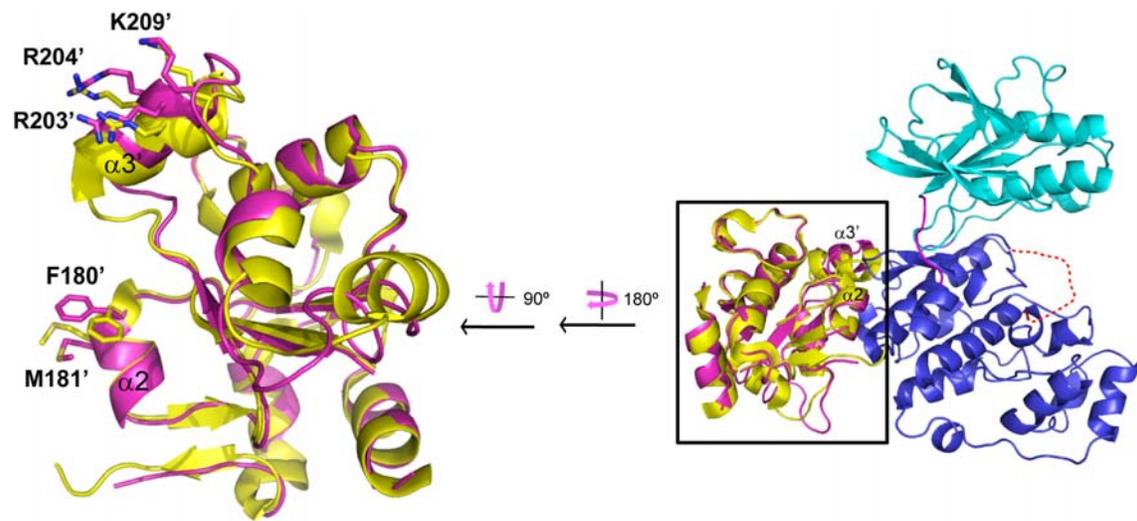


Fig. S1. Comparison of the uncomplexed KBD^{MKP5} with KBD^{MKP5} from the p38α-KBD^{MKP5} complex. The uncomplexed KBD^{MKP5} (PDB ID: 2OUC) was shown in yellow, and the p38α-KBD^{MKP5} complex is colored the same as that in Fig. 2A.

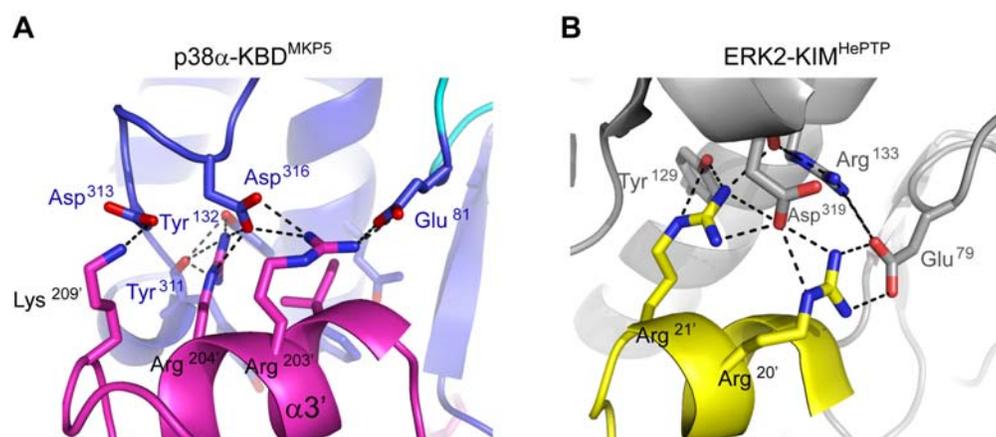


Fig. S2. Comparison of the electrostatic interactions in the CD domains of p38 α and ERK2. (A) Detailed interactions between the basic region of KBD^{MKP5} (magenta) and the CD domain of p38 α (blue). (B) Detailed interactions between the basic region of KIM^{HePTP} (yellow) and the CD domain of ERK2 (grey) (PDB ID: 2GPH). Ion-pair and hydrogen-bonding interactions are indicated by black dashed lines. Helix $\alpha 3'$ and the following $\alpha 3'$ - $\alpha 3$ loop in the KBD^{MKP5} make contacts with the CD domain of p38 α , an acidic patch involving residues Asp³¹³, Asp³¹⁵, Asp³¹⁶ and Glu⁸¹ (A). The side chain of Arg^{203'} in KBD^{MKP5} forms four hydrogen bonds with the carboxylates of Asp³¹⁶ and Glu⁸¹ in p38 α , and the adjacent Arg^{204'} further coordinates residues Asp³¹⁶, Tyr¹³² and Tyr³¹¹ of p38 α . Such a massive electrostatic network is similar to that observed in the structure of ERK2 bound with KIM^{HePTP} (1), where Arg20' and Arg21' of HePTP form extensive interactions with the CD domain of ERK2 (B). This finding supports the notion that the CD domains of both p38 α and ERK2 play a crucial role in coordinating the basic residues in the KIM sequences of their cognate protein partners (2).

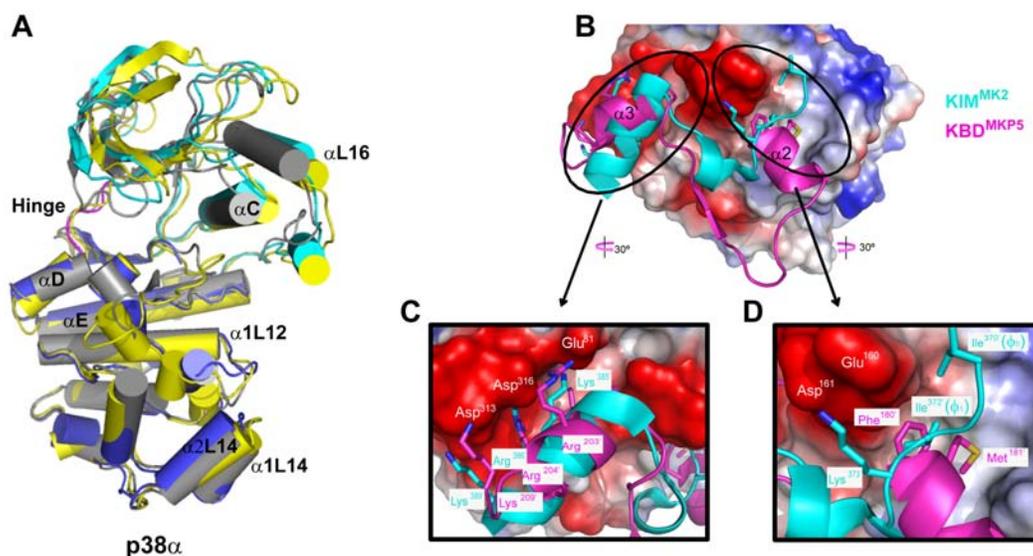


Fig. S3. Comparison of the complex structures of p38 α -KBD^{MKP5} and p38 α -MK2. (A)

Comparison of the p38 α conformations in the p38 α -KBD^{MKP5} and p38 α -MK2 complexes with the uncomplexed p38 α by superimposing the corresponding C α atoms within C-lobe.

The p38 α N-lobe and C-lobe from the p38 α -KBD^{MKP5} complex are respectively shown in cyan and blue, the p38 α molecule from the p38 α -MK2 complex (PDB ID: 2OZA) in grey, and the uncomplexed p38 α (PDB ID, 1P38) in yellow. (B) Comparison of the binding

modes of KBD^{MKP5} (magenta) and MK2 (cyan) to p38 α . For clarity, only residues Phe¹⁸⁰-Lys²⁰⁹ of KBD^{MKP5} and Ile³⁷⁰-Ala³⁹⁰ of MK2 are shown. Detailed electrostatic

interactions and hydrophobic interactions are illustrated in panels (C) and (D), respectively.

The p38 α -KBD^{MKP5} complex is the second structure to be reported on a protein-protein interaction including docking in the CD domain of the MAPK, the first being a complex between p38 α and a protein substrate, MK2 (3). Although the binding of KBD^{MKP5} and MK2 both induced a 5-6° rigid rotation of p38 α N-lobe with respect to its C-lobe, the

resulted p38 α conformations are different (A). An overlay of p38 α in complex with KBD^{MKP5} and that with MK2 shows similar electrostatic contacts in the CD domain, yet different hydrophobic contacts in the docking groove (B). The basic region (Pro^{381'}-Ala^{390'}) in the KIM sequence of MK2 adopts a helical conformation, which resembles that of helix $\alpha 3'$ in KBD of MKP5 (C). Despite a little shift between the two helices, the basic residues on these helices contact the acidic residues in the CD domain of p38 α in a very similar fashion. However, the unique interaction of Lys^{373'} from the MK2 KIM sequence with the p38 α ED site (Glu¹⁶⁰ and Asp¹⁶¹) is not observed in the p38 α -KBD^{MKP5} complex (D). In addition, the reversed Φ_A -X- Φ_B motif (Lue^{372'}-Lys^{371'}-Val^{370'}) of MK2 binds to the p38 α hydrophobic groove extensively; however, in p38 α -KBD^{MKP5} complex, only the Φ_A pocket on p38 α is bound by Phe^{180'} and Met^{181'} from KBD^{MKP5} whereas the Φ_B pocket is unoccupied (D). Therefore, the binding modes of KBD^{MKP5} and MK2 to p38 α are distinct.

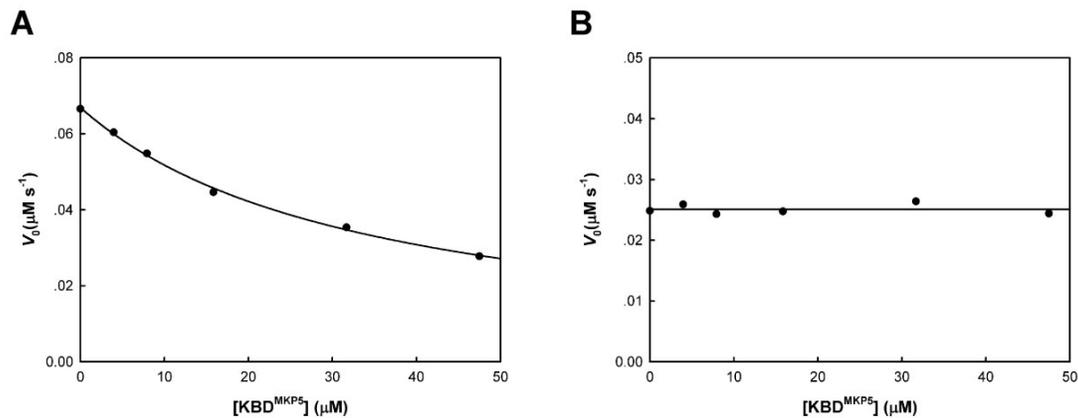


Fig. S4. Effect of KBD^{MKP5} on the kinase activity of phosphorylated p38α toward different substrates. (A) The effect of KBD^{MKP5} on the activity of phosphorylated p38α towards KIM- containing protein substrate ATF2Δ109. The assay was performed in the presence of 32 nM phosphorylated p38α, 4 μM ATF2Δ109, 1 mM ATP, and various concentrations of KBD^{MKP5}. (B) The effect of KBD^{MKP5} on the activity of phosphorylated p38α towards non-KIM containing substrate EGFR peptide, a substrate that does not contain a KIM. The assay was performed in the presence of 13 nM phosphorylated p38α, 100 μM EGFR peptide, 1 mM ATP, and various concentrations of KBD^{MKP5}.

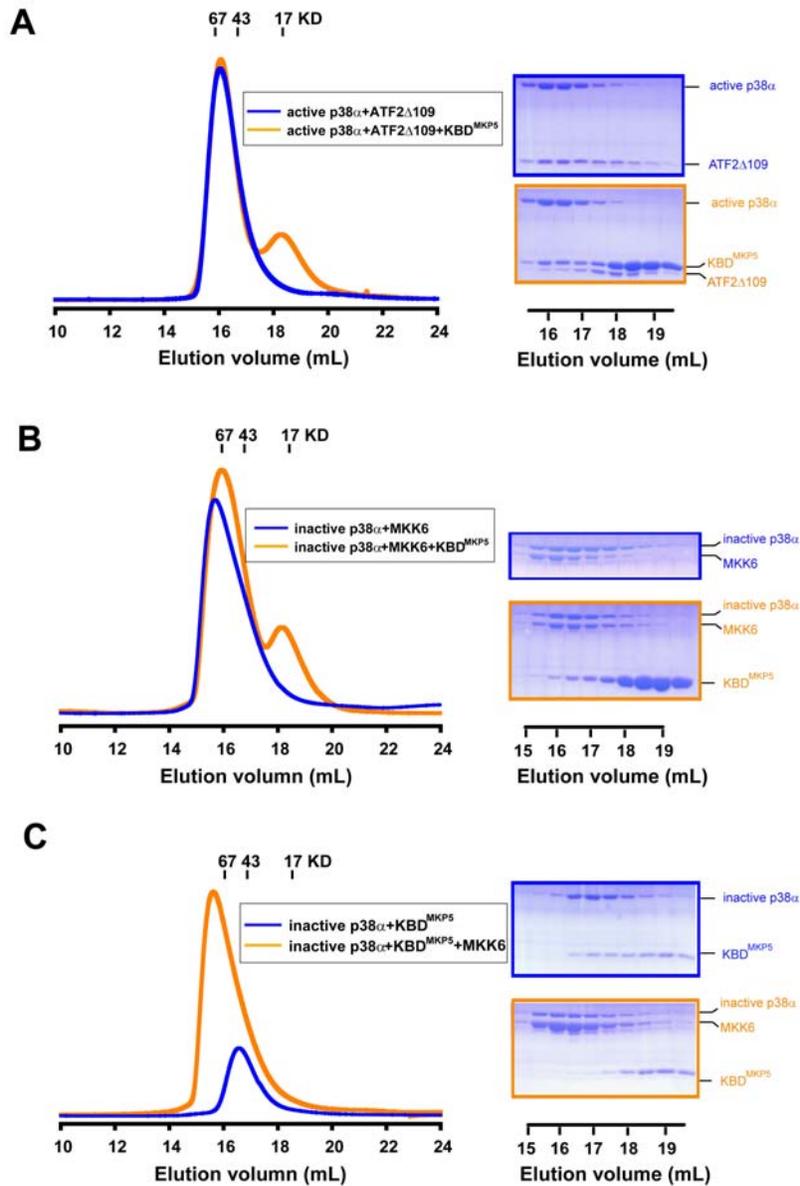


Fig. S5. Effect of KBD^{MKP5} on the interaction of p38 α with different cognate partners.

(A) The effect of KBD^{MKP5} on the interaction of phosphorylated p38 α with its protein substrate ATF2 Δ 109. The mixture of phosphorylated p38 α and ATF2 Δ 109 (ratio, 1:1.5) or that of phosphorylated p38 α , ATF2 Δ 109 and KBD^{MKP5} (ratio, 1:1.5:6) was subjected to gel

filtration analysis. **(B)** The effect of KBD^{MKP5} on the interaction of unphosphorylated p38 α with its upstream kinase MKK6. The mixture of phosphorylated p38 α and MKK6 (ratio, 1.5:1) or that of phosphorylated p38 α , MKK6 and KBD^{MKP5} (ratio, 1.5:1:8) was subjected to gel filtration analysis. **(C)** The effect of MKK6 on the interaction of unphosphorylated p38 α with KBD^{MKP5}. The mixture of phosphorylated p38 α and KBD^{MKP5} (ratio, 1:2) or that of phosphorylated p38 α , KBD^{MKP5} and MKK6 (ratio, 1:2:4) was subjected to gel filtration analysis.

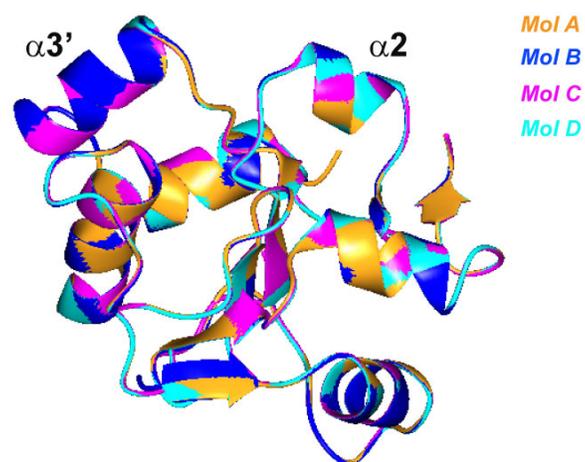


Fig. S6. Superimposition of the four molecules within the asymmetric unit of the KBD^{MKP7} structure.

MKP5	Phospho-substrate	k_{cat}	K_m	k_{cat}/K_m
		s^{-1}	μM	$(\mu M^{-1}s^{-1})$
Wild-type	ERK2	0.577 ± 0.011	1.74 ± 0.12	0.332 ± 0.030
	p38 α	0.574 ± 0.019	0.074 ± 0.013	7.76 ± 1.55
$\Delta N319$	ERK2		>10.0	0.0017 ± 0.0001
	p38 α	0.384 ± 0.03	20.47 ± 3.34	0.019 ± 0.005

Table S1. Kinetic parameters of full-length MKP5 and its catalytic domain toward phosphorylated ERK2 or p38 α .

The values of k_{cat} and K_m for ERK2 were determined to be $0.577 \pm 0.011 s^{-1}$ and $1.74 \pm 0.12 \mu M$, respectively. The k_{cat} / K_m value for the MKP5-catalyzed dephosphorylation of ERK2 can then be calculated as $0.332 \pm 0.030 \mu M^{-1}s^{-1}$, which is more than 20-fold lower than that for the MKP5-catalyzed p38 α dephosphorylation ($7.76 \pm 1.55 \mu M^{-1}s^{-1}$). These data are consistent with the previous findings that MKP5 preferentially recognizes and dephosphorylates p38 α , whereas MKP3 may selectively down-regulate the ERK2 signaling pathway.

Table S2. Kinetic parameters of full-length MKP5 and its mutants with phosphorylated p38 α as substrate.

MKP5	k_{cat}	K_{m}	$k_{\text{cat}}/K_{\text{m}}$
	s^{-1}	μM	$\mu\text{M}^{-1}\text{s}^{-1}$
Wild type	0.574 \pm 0.019	0.074 \pm 0.013	7.76 \pm 1.55
F180A	0.664 \pm 0.031	0.360 \pm 0.063	1.84 \pm 0.40
M181A	0.402 \pm 0.015	0.118 \pm 0.022	3.41 \pm 0.77
F180A&M181A	0.613 \pm 0.023	0.675 \pm 0.070	0.908 \pm 0.134
F180D	0.590 \pm 0.025	1.02 \pm 0.10	0.578 \pm 0.082
M181D	0.612 \pm 0.013	0.63 \pm 0.04	0.971 \pm 0.082
F180D&M181D	0.441 \pm 0.022	1.87 \pm 0.25	0.236 \pm 0.044
I200A	0.818 \pm 0.019	0.308 \pm 0.029	2.66 \pm 0.29
R203A	0.851 \pm 0.063	1.40 \pm 0.27	0.608 \pm 0.158
R204A	0.813 \pm 0.019	2.07 \pm 0.11	0.392 \pm 0.027
R203A&R204A	0.884 \pm 0.031	16.61 \pm 1.25	0.053 \pm 0.006
K209A	0.798 \pm 0.034	0.456 \pm 0.07	1.75 \pm 0.33
ΔN319	0.384 \pm 0.03	20.47 \pm 3.34	0.019 \pm 0.005
I210A (Φ_{A})	0.510 \pm 0.004	0.052 \pm 0.003	9.81 \pm 0.66
V212A (Φ_{B})	0.560 \pm 0.011	0.073 \pm 0.008	7.67 \pm 0.92

Table S3. Kinetic parameters of MKP7 (residues 5 to 303) and its mutants with phosphorylated p38 α as substrate.

MKP7 (5-303)	k_{cat}	K_{m}	$k_{\text{cat}}/K_{\text{m}}$
	s^{-1}	μM	$(\mu\text{M}^{-1}\text{s}^{-1})$
Wild type	0.065 \pm 0.019	0.051 \pm 0.013	1.27 \pm 0.27
F34A	0.060 \pm 0.003	0.104 \pm 0.023	0.577 \pm 0.151
V35A	0.062 \pm 0.002	0.081 \pm 0.016	0.765 \pm 0.181
F34A&V35A	0.065 \pm 0.001	0.141 \pm 0.020	0.463 \pm 0.069
L53A	0.066 \pm 0.002	0.097 \pm 0.018	0.680 \pm 0.153
R56A	0.060 \pm 0.002	0.165 \pm 0.029	0.363 \pm 0.073
R57A	0.061 \pm 0.002	0.321 \pm 0.050	0.190 \pm 0.035
K62A	0.064 \pm 0.002	0.118 \pm 0.014	0.542 \pm 0.080
Δ N155	0.045 \pm 0.001	0.500 \pm 0.090	0.090 \pm 0.020
V63A (Φ_{A})	0.064 \pm 0.002	0.050 \pm 0.009	1.28 \pm 0.25
I65A (Φ_{B})	0.060 \pm 0.001	0.056 \pm 0.007	1.07 \pm 0.15

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