SUPPORTING INFORMATION

Structural and dynamic insights into the mechanism of allosteric signal transmission in ERK2-mediated MKP3 activation

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Experimental Procedures.

Assays for protein-protein interaction. The interactions of ERK2 with the ΔN151, CD and KBD domains of MKP3 were examined by gel filtration analyses using a Superdex-75 10/300 column on an ÄKTA FPLC (GE Healthcare). The column was equilibrated with a buffer containing 10 mM HEPES, pH 7.5, 150 mM NaCl, and 2 mM dithiothreitol (DTT), and calibrated with molecular mass standards. Samples of individual proteins and indicated mixtures (500 μl each) were loaded to the Superdex-75 column and then eluted at a flow rate of 0.5 ml/min. Fractions of 0.5 ml each were collected, and aliquots of relevant fractions were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) followed Coomassie Blue staining.

Figures.

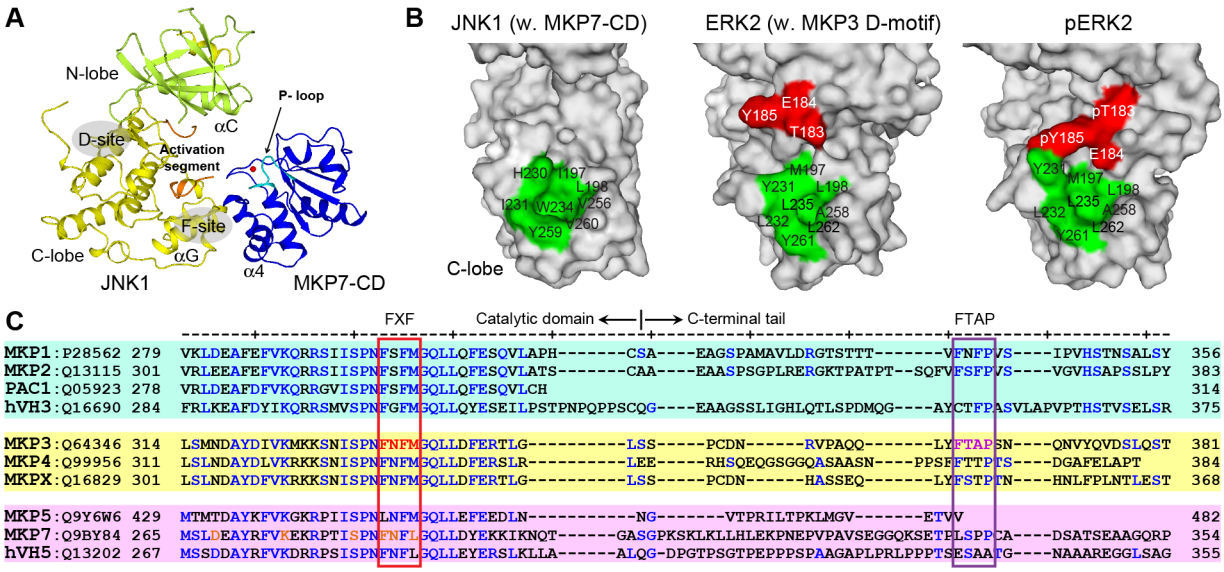

Figure S1. Conservation of F-site and FXF-motif. (A) Structure of JNK1 in complex with MKP7-CD (PDB entry 4YR8). The N-lobe and C-lobe of JNK1 are respectively colored in lemon and yellow, and MKP7-CD is shown in blue. (B) Comparisons of the F-site regions of JNK1 (in complex with MKP7-CD), ERK2 (in complex with MKP3 D-motif, PDB entry 2FYS) and pERK2 (PDB entry 2ERK). Residues forming the F-sites are colored in green, and the 183TEY185 motif in the activation segment of ERK2 is highlighted in red. (C) Sequence alignment of the C-terminal part of ten MKPs. The FXF motif and the FTAP motif are respectively boxed in red and purple, and residues of MKP7 involved in recognition of JNK1 are highlighted in orange.

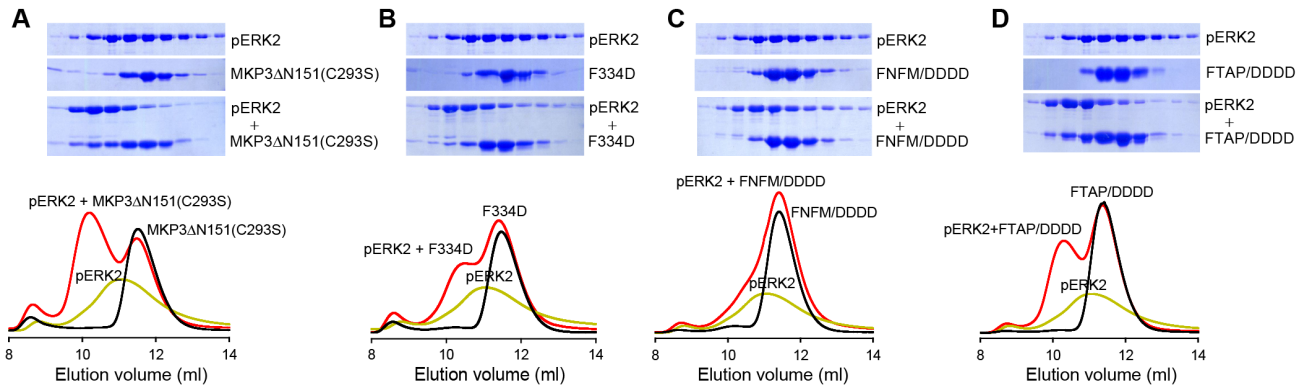

Figure S2. Gel filtration results for different mutations of MKP3ΔN151 and phosphorylated ERK2. (A) MKP3ΔN151(C293S) wildtype exhibits interaction with pERK2, (B-C) mutations of FXF motif disrupts interaction with pERK2, (D) mutation of FTAP residues retains interaction with pERK2.

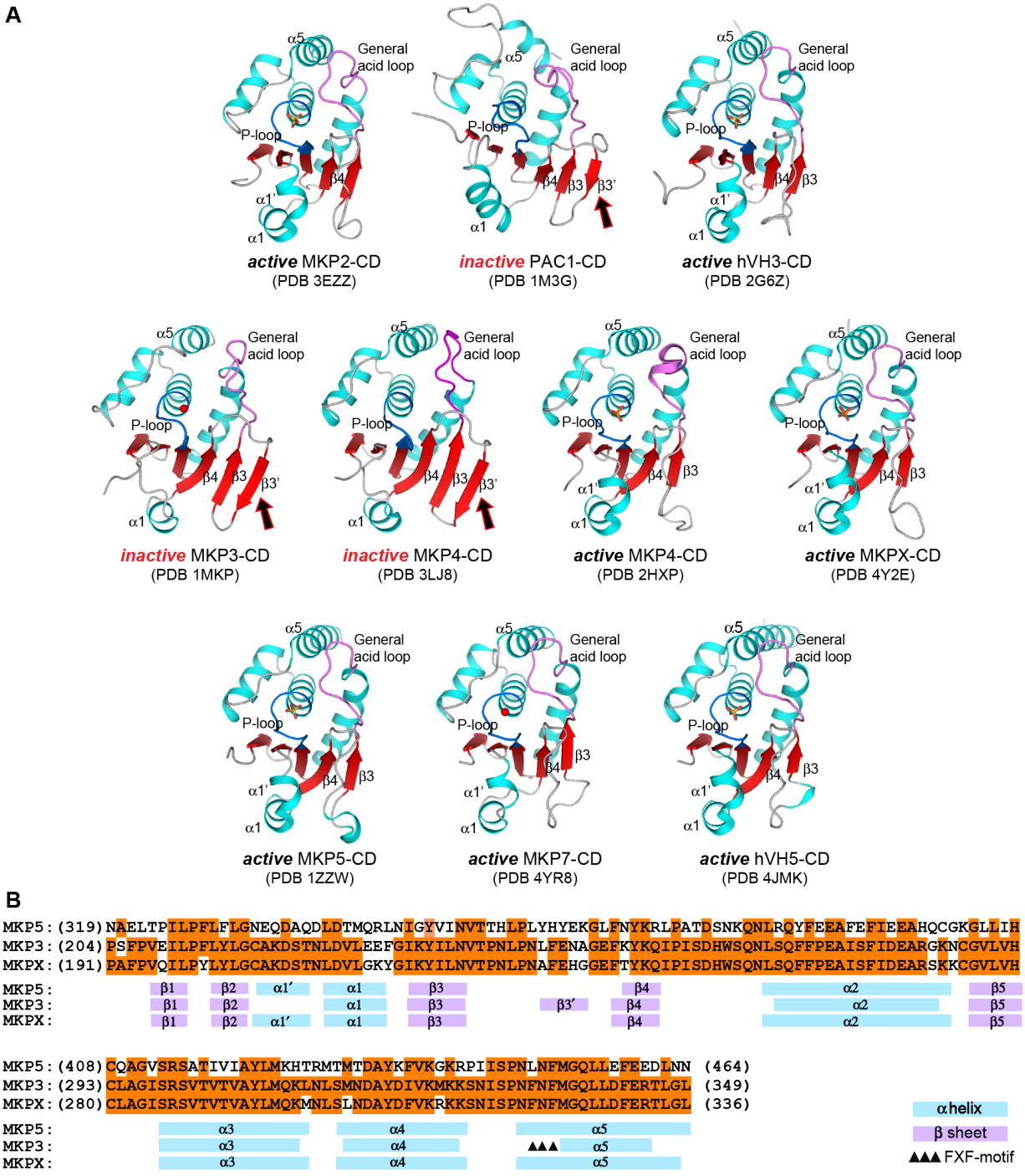

Figure S3. Family-wide structure comparisons of the MKPs catalytic domains. (A) Ribbon diagrams of the structures of human MKP-CDs. Secondary structural elements are labeled. The bound sulfate ions and phosphate ion are presented as a stick model, and the chloride ions bound in the active site are shown in red. The D-loop and P-loop conformations are

indicated under each structure. Currently, the Protein Data Bank contains two crystal structures of MKP4-CD (ligand-bound form, PDB entry 2HXP and ligand free form, PDB entry 3LJ8), which exhibit significant structural divergence. The ligand-bound form of MKP4-CD has the canonical P-loop and closed D-loop conformation, whereas the ligand-free form of MKP4-CD exhibits the inactive P-loop and open D-loop conformation. Inspection of all MKP-CD structures available at the Protein Data Bank reveals that all closed D-loop structures share a tightly bound ‘‘negatively charged ion’’ coordinated by the amide atoms the main-chain in the active-site P-loop and the guanidino moiety of the Arg. In contrast, in structures with an open or atypical D-loop conformation this substrate-mimic ion was not observed or was significantly displaced, suggesting that it is a key part of the closure mechanism.

(B) Sequence alignment of MKP5-CD, MKP3-CD and MKPX-CD. The secondary structure assignments of MKP5-CD, MKP3-CD and MKPX-CD are shown below the sequences. The catalytic domain of MKPX shares 89% and 48% sequence identity with that of MKP3 and MKP5, respectively. Although the catalytic domains of MKPX and MKP3 have much greater sequence similarity, the MKPX-CD structure is more similar to MKP5-CD (r.m.s.d. of 0.7 Å over 123 common Cα atoms) than to MKP3-CD (r.m.s.d. of 1.2 Å over 117 common Cα atoms). Indeed, the isolated catalytic domain of MKPX has all structural features observed in MKP5-CD, suggesting that it is in the active conformation. In summary, the comprehensive analysis of the MKP subfamily reveals that the inducibly active MKPs can adopt both active and inactive conformations in the absence of MAPK substrate. The apparent difference in the requirement of substrate binding for the activating conformational transition may be due to different equilibrium between the active and inactive conformations among MAPKs, or it may simply reflect the different crystallization conditions.

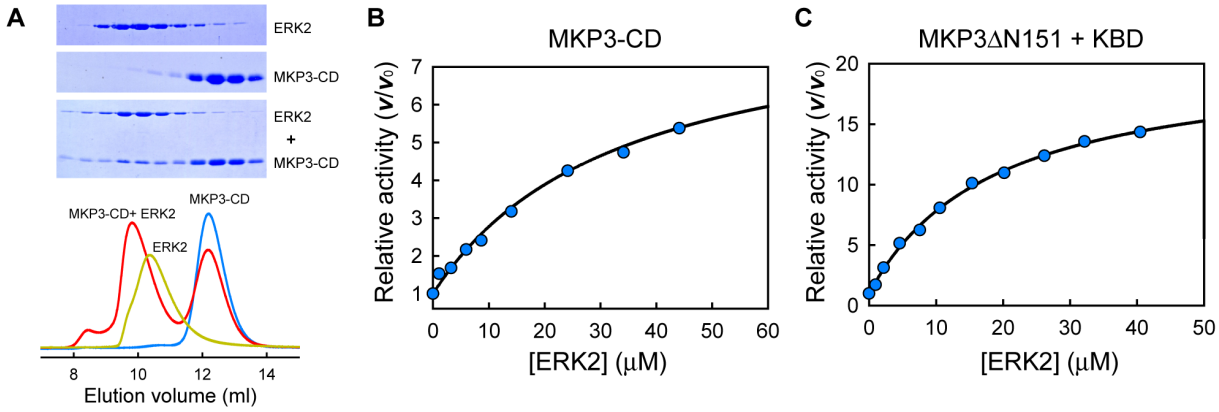

Figure S4. Binding and allosteric activation of MKP3 by ERK2. (A) Gel filtration result of MKP3-CD and unphosphorylated ERK2. (B) Relative activity of hydrolysis of *p*NPP by MKP3-CD in the presence of the indicated concentrations of unphosphorylated ERK2 compared to without ERK2. (C) Relative activity of hydrolysis of *p*NPP by MKP3ΔN151 with versus without ERK2, in the presence of 11 μM KBD domain (MKP3, 1-151).

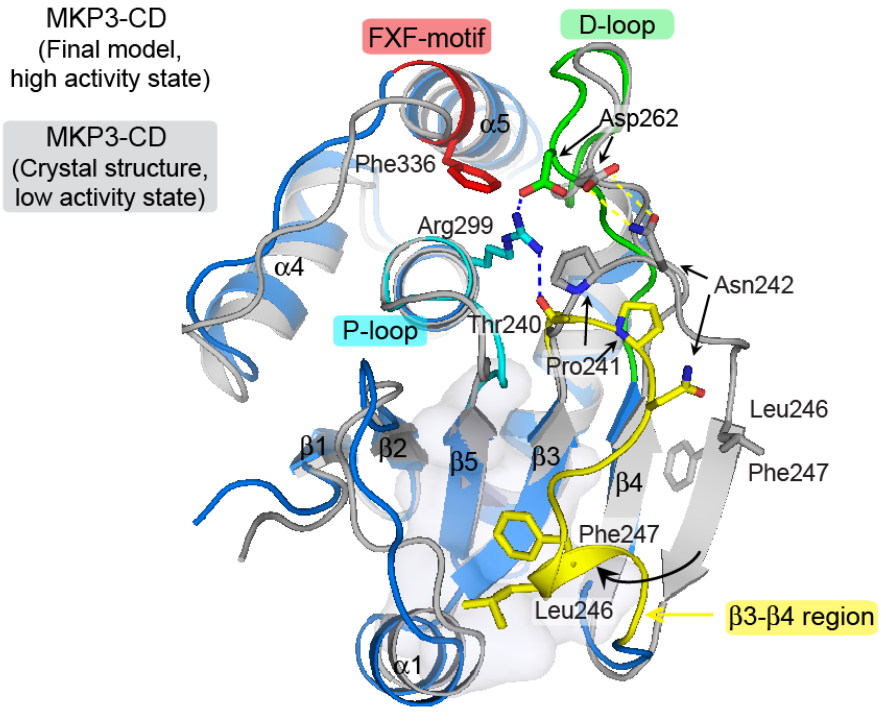

Figure S5. Comparison of the crystal structure and the final activated model of MKP3-CD. The MKP3-CD crystal structure is colored in gray. Selected residues including Asp262, Pro241, Asn242, Leu247 and Phe247 are highlighted with sticks.

The final model of MKP3-CD (ERK2-bound) is colored in marine with the essential loops, e.g. D-loop, P-loop, β3-β4 region and FXF-motif colored as indicated. Specifically, the D-loop (residues 259-268) is highlighted in green, with Asp262 shown in sticks; the β3- β4 region (residues 240 to 250) is highlighted in yellow, with Thr240, Pro241, Asn242, Leu246 and Phe247 shown in sticks; the P-loop (residues 292-299) is highlighted in cyan, with Arg299 shown in sticks; the FXF-motif (residues 334 to 336) is highlighted in red, with Phe336 shown in sticks.

The Leu246 and Phe247 residues, located in the original β3' strand of MKP3-CD crystal structure, are inserted into a hydrophobic pocket formed by residues from α1, β3 and β5, as shown in surface representation with 80% transparency.

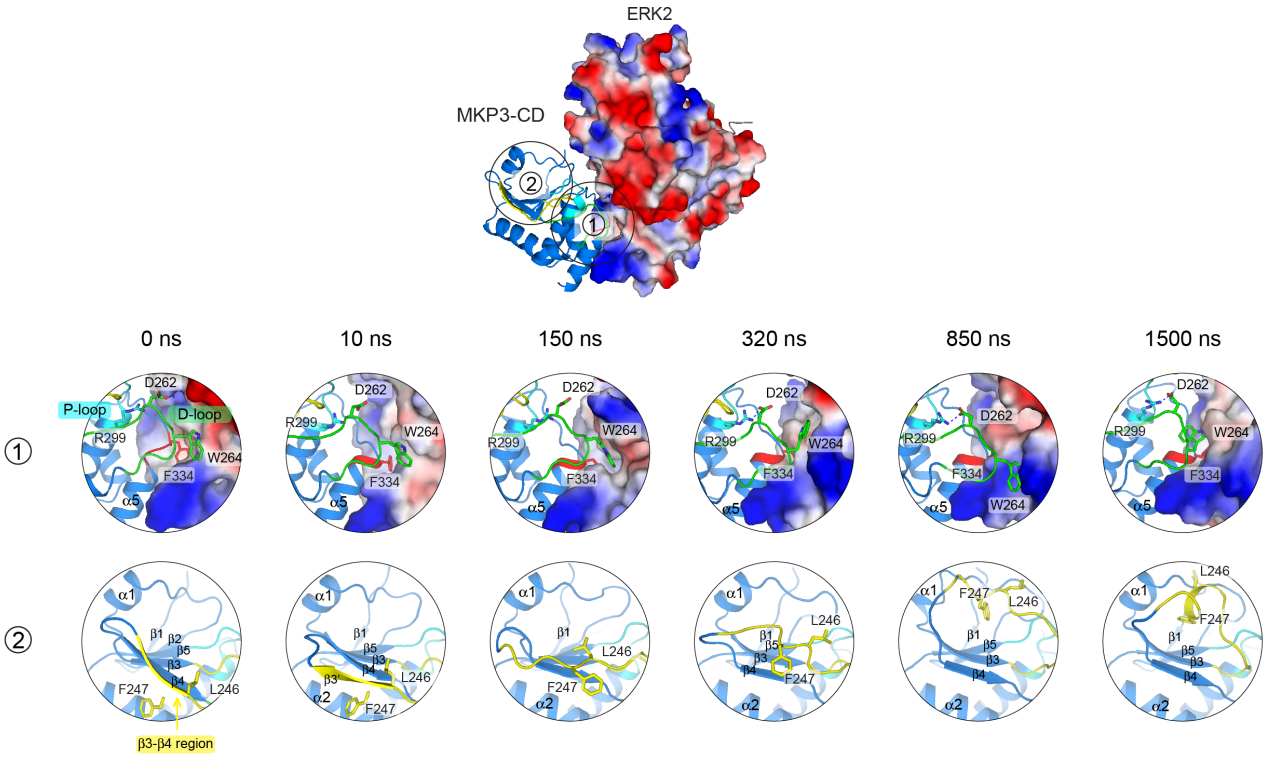

Figure S6. Time-dependent detailed view of MKP3-CD conformational change.

Upper panel: Initial model of ERK2-MKP3-CD complex. Detailed view of the two indicated areas are shown below.

Lower panel: ① Detailed view of the interaction surface. Residues Asp262, Trp264 on the D-loop, Arg299 on the P-loop and Phe334 from FXF motif are shown in sticks. Polar interactions are shown as dashed lines. ② Detailed view of the β3-β4 region. Residues Leu246 and Phe247 from the β3' strand are shown in sticks.

Tables

Table S1. Sequence identity of MKP catalytic domains.

MKP1	PAC1	MKP2	hVH3	MKP3	MKPX	MKP4	hVH5	MKP5	MKP7	
***	75	86.1	66	46.5	46.5	45.8	43.8	42.4	43.8	MKP1
	***	72.9	59	47.2	48.6	47.2	44.4	43.1	45.1	PAC1
		***	66	47.2	47.2	47.9	42.4	42.4	42.4	MKP2
			***	44.4	43.1	44.4	36.1	35.4	41	hVH3
				***	88.9	80.6	43.8	47.9	41	MKP3
					***	81.9	45.1	47.9	42.4	MKPX
						***	43.1	47.2	42.4	MKP4
							***	42.4	75.7	hVH5
								***	41.7	MKP5
									***	MKP7

Calculated based on the sequences corresponding to the catalytic domain of MKP3 (residues 204-348).

Vertical lines: separation of 10 mammalian MKPs into three groups.

Red fill highlight: identities >50%.

Movies.

The movie legends are provided below. All states were collected after each ns, and all movies are generated by smoothing over every 2 frames.

Movie S1. MD simulation on the apo-MKP5-CD (1000 ns)

MKP5-CD is shown in cartoon and colored silver. The D-loop (residues 374-383) is highlighted in green, with Asp377 shown in sticks; the β3- β4 region (residues 355 to 365) is highlighted in yellow; the P-loop (residues 407-414) is highlighted in cyan, with Arg414 shown in sticks; the FXF-motif (residues 449 to 451) is highlighted in red, with Phe451 shown in sticks.

Movie S2. MD simulation on the apo-MKP3-CD (1000 ns)

MKP3-CD is shown in cartoon and colored silver. The D-loop (residues 259-268) is highlighted in green, with Asp262 shown in sticks; the β3- β4 region (residues 240 to 250) is highlighted in yellow; the P-loop (residues 292-299) is highlighted in cyan, with Arg299 shown in sticks; the FXF-motif (residues 334 to 336) is highlighted in red, with Phe336 shown in sticks.

Movie S3. MD simulation on the ERK2-MKP3-CD complex (1500 ns, view zoomed on MKP3-CD)

ERK2 is shown in cartoon and colored silver. The F-site of ERK2 is shown in surface representation, which includes residues 194 to 202 and residues 227 to 266.

MKP3-CD is shown in cartoon and colored blue. The D-loop (residues 259-268) is highlighted in green, with Asp262 and Trp264 shown in sticks; the β3- β4 region (residues 240 to 250) is highlighted in yellow, with Pro241 and Asn242 shown in sticks; the P-loop (residues 292-299) is highlighted in cyan, with Arg299 shown in sticks; the FXF-motif (residues 334 to 336) is highlighted in red, with Phe334 shown in sticks.

Movie S4. MD simulation on the ERK2-MKP3-CD complex (1500 ns, view zoomed on interaction surface)

The coloring scheme is identical with Movie S3.