

Supplementary Information

An aptamer to the MAP kinase insert region

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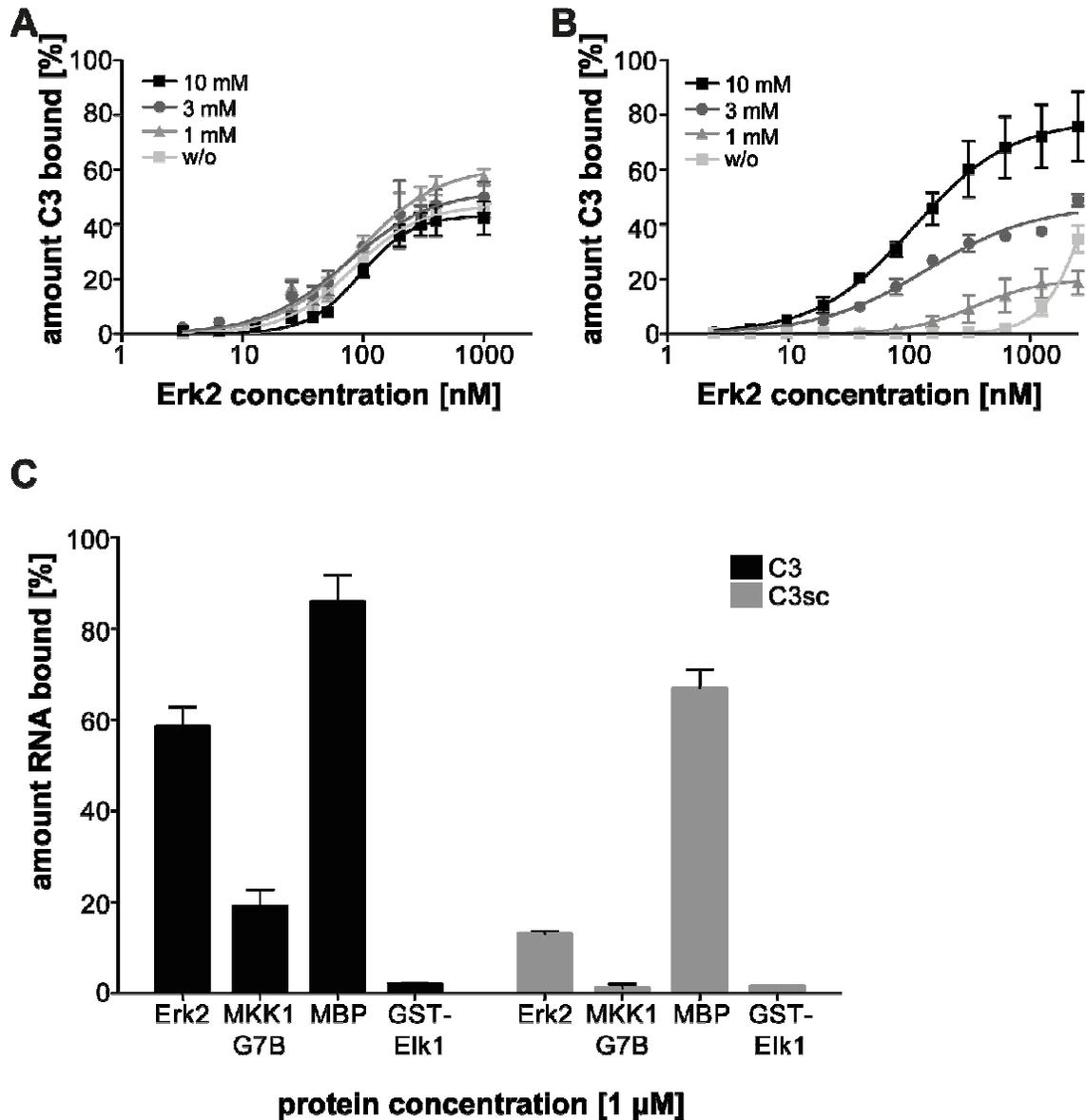
Supplementary Table 1: K_D values of C3 towards Erk2 under varying ionic conditions [nM].

	w/o	1 mM	3 mM	10 mM
PBS	81 ± 11	92 ± 16	75 ± 20	95 ± 12
Hepes	> 1000	346.0 ± 118	142 ± 27	109 ± 25

w/o: without additional $MgCl_2$

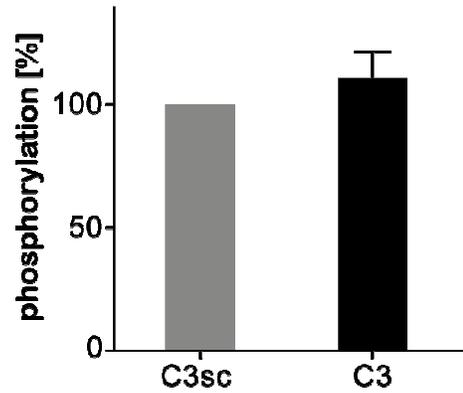
		<u>gly-rich loop</u>
Erk2	MAAAAAAGAG-----PEMVRGQVFDVGPRTNLSYI	<u>GEGAYGMVCSAY</u>
Erk1	MAAAAAQGGGGEGEPRRTEGVGPGVPEVEMVKGQPF	<u>DVGPRTQLQYIGEGAYGMVSSAY</u>
p38alpha	MSQERPTFYR-----QELNKTIEWEVPERYQNLSPV	<u>GSGAYGSVCAAF</u>
JNK2alpha	MDSKCDSQFYS-----VQVADSTFTVLKRYQQLKP	<u>IGSGAQGIVCAAF</u>
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Erk2	DNVNKRVAIKKIS-PFEHQTYCQRTLREIKILLRFRH	ENIIGINDIIR-APTIEQMKDV
Erk1	DHVRKTRVAIKKIS-PFEHQTYCQRTLREIQILLRFR	HENVIGIRDILR-ASTLEAMRDV
p38alpha	DTKTGLRVAVKKLSRPFQSIHAKRTYRELRLKHKH	ENVIGLLDVFTPARSLEEFNDV
JNK2alpha	DTVLGINVAVKKLSRPFQNTTHAKRAYRELVLLKCV	NHKNIISLLNVFTPQKTLEEFQDV
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Erk2	YIVQDLMETDLYKLLKTOHLSNDHICYFLYQILRGL	KYIHSANVLHRDLKPSNLLINTTC
Erk1	YIVQDLMETDLYKLLKSQQLSNDHICYFLYQILRGL	KYIHSANVLHRDLKPSNLLINTTC
p38alpha	YLVTHLMGADLNNIVKCKLTDHVQFLIYQILRGL	KYIHSADIIHRDLKPSNLAVNEDC
JNK2alpha	YLMELMDANLCQVIH-MELDHERMSYLLYQMLCGI	KHLHSAGIIHRDLKPSNIVVKSDC
		<u>Activation segment</u>
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Erk2	DLKICDFGLARVADPDHDHTGFLEYVATRWRAP	EIMLNSKGYTKSIDIWSVGCILAEM
Erk1	DLKICDFGLARVADPDHDHTGFLEYVATRWRAP	EIMLNSKGYTKSIDIWSVGCILAEM
p38alpha	ELKILDFGLARHTDEM-----TGYVATRWRAP	EIMLNSKGYTKSIDIWSVGCILAEM
JNK2alpha	TLKILDFGLARVADPDHDHTGFLEYVATRWRAP	EIMLNSKGYTKSIDIWSVGCILAEM
		<u>MAP kinase insert domain</u>
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Erk2	LSNRPIFPGKHYLDQLNHILGILGSPSQEDLN	CIINLKARNYLLSLPHKNKVPWNRIFFP-
Erk1	LSNRPIFPGKHYLDQLNHILGILGSPSQEDLN	CIINMKARNYLLSLPHKNKVPWNRIFFP-
p38alpha	LTGRTLFPDTHIDQLKLILRLVGTGAEELKKI	SS ESARNYIQSLTQMPKMNFAVFI-
JNK2alpha	VKGCVIFQDTHIDQWNKVIEQLGTPSAEFM	KLQP -TVRNYVENRPKYPGIKFEELFPD
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Erk2	-----NADSKALDLLDKMLTFNPHKRIEVEQ	ALAHYPYEQYYDPSD-EPVIAEAP
Erk1	-----KSDSKALDLLDRMLTFNPNKRITVEE	ALAHYPYEQYYDPTD-EPVAEEP
p38alpha	-----GANPLAVDLEKMLVLDSDKRITAAQ	ALAHAYFAQYHDPDD-EPVADP-
JNK2alpha	WIFPSESERDKIKTSQARDLLSKMLVIDPDKR	ISVDEALRHPYITVWYDPAEAEAPPQI
		<u>DRS</u>
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Erk2	FKFDMELDDLPEKELKELIFEETARFQPGYRS	-----
Erk1	FTFAMELDDLPEKELKELIFEETARFQPGVLEAP	-----
p38alpha	YDQSFESRDLLIDEWKSLTYDEVISFVPPPLD	QEEMES-----
JNK2alpha	YDAQLEEREHAIEEWKELIYKEMDWEERSKNG	VVKDQPSDAAVSSNATPSQSSSINDIS
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Erk2	-----	
Erk1	-----	
p38alpha	-----	
JNK2alpha	SMSTEQTLASDTSLLDASTGPLEGCR	

Supplementary Figure 1: Sequence homology between distinct MAP kinases. Alignment of the MAP kinases Erk2 (P28482), Erk1 (P28482), p38 α (Q16539), and JNK2 α (P45984). Identical amino acids are underlined in grey. The kinase domain is annotated in brackets. The glycine-rich loop, the activation segment and the D-site recruitment site (DRS) residue Asp319, are highlighted in green, red and blue, respectively. The MAP kinase insert is marked in orange. Amino acids found to be critical for C3 binding (Tyr261 and Ser264) are shown in bold orange.

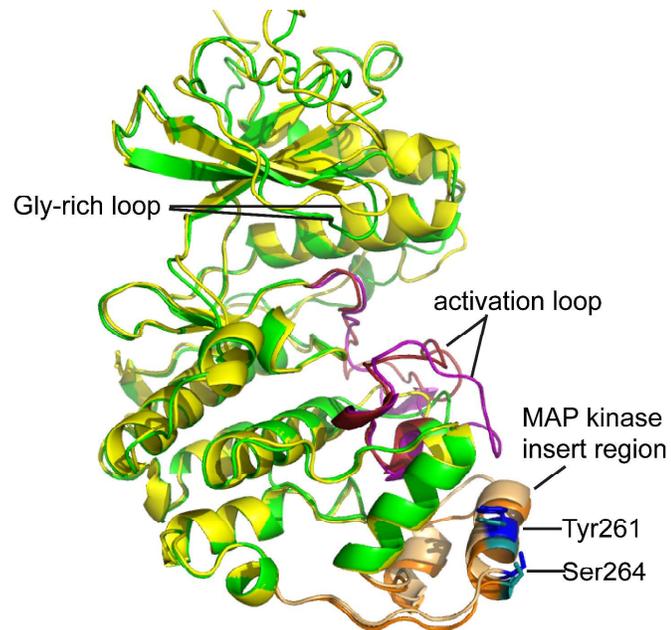


Supplementary Figure 2: C3 binds to Erk2 under a variety of ionic conditions. Filter retention assay of the amount of radioactively labelled C3 bound to Erk2 in PBS (A) or HEPES (B) buffer with varying MgCl₂ concentrations. The K_D values are listed in Supplementary Table 1. Experiments were performed at least twice. In PBS buffer, C3 binds with similar affinity to Erk2 in the absence or presence of up to 10 mM MgCl₂. C3 tolerates the absence of monovalent cations in HEPES buffer. However, its affinity for Erk2 decreased when the MgCl₂ concentration was reduced to 1 mM or beneath. w/o: without additional MgCl₂. C) Additional specificity test were performed by incubating C3 or control RNA in the presence of 1 μM Erk2, 1 μM MKK1 G7B, 10 μM MBP or 1 μM Elk1 in kinase assay buffer (20 mM

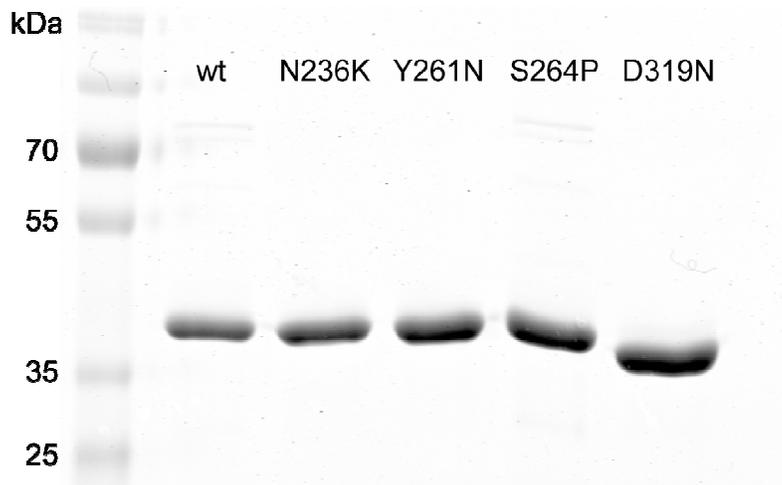
Hepes, pH 7,4, 10 mM MgCl₂). After an incubation of 15 min at 30°C, filter retention assays were performed as described in the Material & Methods section. Under these conditions, C3 showed slightly binding to MKK1 G7B, whereas no binding to Elk1 (GST-Elk1) was observed. Binding of C3 and control RNA to MBP was also detected. This most likely is due to unspecific binding mediated by electrostatic interactions of poly-anionic RNA with highly positively charged MBP under the applied conditions



Supplementary Figure 3: Kinase assays additional data. C3 does not inhibit MKK1 G7B kinase activity. In order to rule out that C3 affects the catalytic activity of MKK1, additional kinase assays were performed. In these assays, 1 μ M MKK1 G7B was incubated with 10 μ M MBP in reaction buffer (20 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 100 μ M ATP). 12.5 μ M C3 or control RNA was added to the mixture and incubated for 15 minutes at 30°C. Reactions were processed as described in the Material and Methods section. As the readout, autophosphorylation of MKK1 G7B was used. Under the tested conditions, C3 did not affect MKK1 phosphorylation. Experiments were performed three times (mean \pm SEM).



Supplementary Figure 4: Overlay of active and inactive Erk2. Overlay of the cartoon diagram crystal structures of active (PDB 2ERK) and inactive (PDB 1ERK) Erk2. The complete structure of inactive Erk2 and its activation loop sequence and MAP insert region are shown in yellow, purple and orange, respectively. The structure of active Erk2 and its activation loop sequence and MAP kinase insert region are shown in green, red and beige. Ser261 and Tyr264 side chains are shown as sticks. Ser261 and Tyr264 of inactive Erk2 are marked in cyan whereas Ser261 and Tyr264 of active Erk2 are shown in blue.



Supplementary Figure 5: SDS-PAGE analysis of Erk2 and point mutants thereof used throughout this study. 5 μ l of purified proteins were run on a 10% SDS-PAGE gel and stained with Coomassie. Purity of Erk2 wild type and mutant proteins was analysed by SDS-PAGE followed by staining with Coomassie brilliant blue. The purity was determined to be greater than 90%. The gel was scanned using the Odyssey imaging system (LiCor).