Supporting Information

Selective MAP kinases proteolysis determined by activation loop flexibility

Lihi Gur-Arie[1], Maayan Eitan-Wexler[2], Nina Weinberger[2], Ilan Rosenshine[1], Oded Livnah[2]

[1]Department Microbiology and Molecular Genetics, IMRIC, Faculty of Medicine, and [2]Department of Biological Chemistry, Alexander Silverman Institute of Life Sciences, The Wolfson Centre for Applied Structural Biology, The Edmond J. Safra Campus, The Hebrew University of Jerusalem.

Figure S1. NleD ortholog protein sequences. NleD protein sequences, as determined by PSI-Blast search results (NCBI). Sequences were aligned using Clustal Omega (Sievers et al., 2011). The HELLH Zn-metalloprotease motif is encircled in green. An asterisk (*) indicates a fully conserved residue. A colon (:) and a period indicates conservation of strongly similar and weakly similar properties respectively.

Figure S2: Sequence alignment of JNK2, p38a, and ERK2 MAP kinases. The regions which were chosen for replacement are colored as detail below:Marked in yellow: activation loop; bolded: TXY motif- brown: αEF/αF loop -green: L13-αG -blue: MKI. Magenta: part of L16

Table S1– Bacterial strains used in this study. The strain name in our collection is brackets.

Name	Description	Reference or source
E2348/69	EPEC wild-type, strain E2348/69	James Kaper
KB4298	E2348/69 Δ*nleD*::*kan*	(Baruch et al., 2011)
BL21(DE3)	F-, *ompT*, *hsdS*β(rβ-mβ-), *dcm, gal*, (DE3) *tonA*	Novagen
KB4583	*Salmonella enterica* serovar Arizona (SEA) strain CDC346-86 SARC5	ATCC
ICC168 (4368)	*Citrobacter rodentium*	Gad Frankel
Rosetta	*E. coli* strain Rosetta	Novagen

Table S2: Plasmids used in this study

Name	Description	Reference or source	Name in our collection
JNK2	pET28a, bacterial expression vector encoding hexa-His tag JNK2	David Engelberg	pLG4470
p38α	pET28a, bacterial expression vector encoding hexa-His tag p38α	This study	
ERK2	pET15b, bacterial expression vector encoding hexa-His tag ERK2	David Engelberg	pLG4472
NleD	pET28a, bacterial expression vector encoding hexa-His tag truncated *nleD* $_{\text{EPEC 19-232}}$	This study	pLG6728
$ERK_{A\text{-}JNK}$	pET15b, bacterial expression vector encoding ERK2 with JNK2 activation loop (residues 169-188 from JNK2)	This study	pLG6725
$ERK_{A\text{-}p38}$	pET15b, bacterial expression vector encoding ERK2 with p38α activation loop (residues 167-185 from p38α)	This study	pLG6726
$p38_{A\text{-}ERK}$	pET28a, bacterial expression vector encoding p38α with ERK2 activation loop (residues 164-188 from ERK2)	This study	pLG6727
$ERK_{G\text{-}p38}$	pET15b, bacterial expression vector encoding ERK2 with G-helix from p38α (residues 223-229 from p38α)	This study	
$ERK_{G\text{-}JNK}$	pET15b, bacterial expression vector encoding ERK2 with G-helix from JNK2 (residues 225-231 from JNK2)	This study	
$ERK_{G,M,L\text{-}p38}$	pET15b, bacterial expression vector encoding ERK2 with G-helix, MKI and L16 from p38α (residues 223-229, 243-262, 327-333, respectively, from p38α)	This study	
$ERK_{G,M,L\text{-}JNK}$	pET15b, bacterial expression vector encoding ERK2 with G-helix, MKI and L16 from JNK2 (residues from 225-231, 245-262, 342-348, respectively, from JNK2)	This study	
$ERK_{F\text{-}p38}$	pET15b, bacterial expression vector encoding ERK2 with αEF/αF loop from p38α (residues 196-202 from p38α)	This study	
$ERK_{G,F\text{-}p38}$	pET15b, bacterial expression vector encoding ERK2 with αEF/αF loop and G-helix from p38α (residues	This study	

	196-202 and 223-229, respectively, from p38α)		
$ERK_{G,F,M,L\text{-}p38}$	pET15b, bacterial expression vector encoding ERK2 containing ERtrp38+ ERαEF/αFp38	This study	
p38α D316N	pET28a, bacterial expression vector encoding hexa-His tag p38α with D316N	Lab collection	
pET28a	Bacrerial expression vector	Novagen	pLG4483
NleD EPEC	pET28a, bacterial expression vector encoding $nleD_{EPEC}$	This study	pLG4480
pSA10	pKK177-3 derivative containing $lacI^q$. bacterial expression vector.	(Schlosser-Silverman et al., 2000)	1060
NleD EPEC	pSA10, bacterial expression vector encoding $nleD_{EPEC}$	(Baruch et al., 2011)	pKB4345
NleD CR1	pSA10, bacterial expression vector encoding $nleD1_{CR}$	This study	pKB4505
NleD CR2	pSA10, bacterial expression vector encoding $nleD2_{CR}$	This study	pKB4506
NleD SEA	pSA10, bacterial expression vector encoding $nleD_{SEA}$	This study	pSK4611
NleD HD	pSA10, bacterial expression vector encoding $nleD_{HD}$	This study	pSK4702
GST-7Ala-GFP	pGEX2T, bacterial expression vector encoding GST – 7 alanine loop – GFP	This study	pLG5088
GST- $JNK_{169\text{-}192}$-GFP	pGEX2T, bacterial expression vector encoding GST –JNK2 $_{D169 – R192}$ – GFP	This study	pKB4699
GST- $JNK_{176\text{-}192}$-GFP	pGEX2T, bacterial expression vector encoding GST –JNK2 $_{A176 – R192}$ – GFP	This study	pLG5011
GST- $JNK_{180\text{-}192}$-GFP	pGEX2T, bacterial expression vector encoding GST –JNK2 $_{F180 – R192}$ – GFP	This study	pLG5102
GST- $JNK_{176\text{-}188}$-GFP	pGEX2T, bacterial expression vector encoding GST –JNK2 $_{A176 – T188}$ – GFP	This study	pLG5101
GST- $JNK_{180\text{-}186}$-GFP	pGEX2T, bacterial expression vector encoding GST –JNK2 $_{F180 – V186}$ – GFP	This study	pLG5086
GST- $JNK_{182\text{-}188}$-GFP	pGEX2T, bacterial expression vector encoding GST –JNK2 $_{M182 – T188}$ – GFP	This study	pLG5087
GST- ERK_A-GFP	pGEX2T, bacterial expression vector encoding GST–ERK1 $_{183\text{-}217}$ – GFP	This study	pLG5103
JNK2	pGEX2T, bacterial expression vector encoding hexa-His tag JNK2	This study	pLG4901

JNK2 P184E	pGEX2T, bacterial expression vector encoding hexa-His tag JNK2 mutant P184E	This study	pLG5186
p38β	pET28a, bacterial expression vector encoding hexa-His tag p38β	David Engelberg	pLG4495
ERK1	pET28a, bacterial expression vector encoding hexa-His tag ERK1	This study	pLG4774
JNK1	pSRα3, mammalian expression vector encoding HA-JNK1,	Eitan Shaulian	pLG4487
JNK2	pSRα3, mammalian expression vector encoding HA-JNK2	Eitan Shaulian	pLG4488
p38α	pcDNA3, mammalian expression vector encoding HA-p38α	David Engelberg	pLG4683
p38β	pcDNA3, mammalian expression vector encoding HA-p38β	David Engelberg	pLG4684
p38γ	pcDNA3, mammalian expression vector encoding HA-p38γ	David Engelberg	pLG4685
p38δ	pcDNA3, mammalian expression vector encoding HA-p38δ	David Engelberg	pLG4686
ERK1	pcEFL, mammalian expression vector encoding hexa-His ERK1	David Engelberg	pLG4675
ERK2	pcEFL, mammalian expression vector encoding HA-ERK2	David Engelberg	pLG4676
JNK2 D326A	pSRα3, mammalian expression vector encoding HA-JNK2 with D326A mutation	This study	pLG6680
JNK2 E329A	pSRα3, mammalian expression vector encoding HA-JNK2 with E329A mutation	This study	pLG6681
JNK2 E331A	pSRα3, mammalian expression vector encoding HA-JNK2 with E331A mutation	This study	pLG6682
JNK2 D326A/ E329A/ E331A	pSRα3, mammalian expression vector encoding HA-JNK2with the mutations D326A, E329A and E331A	This study	pLG6683

Table S3 – The primers used in this study

Plasmid number	Primer sequence
pLG4480	CAGAATCATATGCGCCCTACGTCCCTC
	AGAATTCAGCTAAAGCAATGGATGCAG
pLG4901	CggatccATGAGCGACAGTAAATGTG
	GGAATTCTCATCGACAGCCTTCAAGG
pLG5011	CAGGGATCCGCGTGCACTAACTTCATGATGACCCCTTACGT GGTGACACGGTACTACCGGATGGTGAGCAAGGGCGAG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pLG5086	CAGGGATCCTTCATGATGACCCCTTACGTGATGGTGAGCAA GGGCGAG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pLG5087	CAGGGATCCATGACCCCTTACGTGGTGACAATGGTGAGCAA GGGCGAG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pLG5088	CAGGGATCCGCAGCTGCGGCCGCAGCTGCAATGGTGAGCA AGGGCGAG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pLG5101	CAGGGATCCGCGTGCACTAACTTCATGATGACCCCTTACGT GGTGACAATGGTGAGCAAGGGCGAG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pLG5102	CAGGGATCCTTCATGATGACCCCTTACGTGGTGACACGGTA CTACCGGATGGTGAGCAAGGGCGAG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pLG5103	CAGGGATCCTGTGATTTCGGCCTGGCCCGGATTGCCGATCC TGAGCATGACCACACCGGCTTCCTGACGGAGTATGTGGCTA CGCGCTGGATGGTGAGCAAGGGCGAG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pSK4702	ATCGGACCCGGGATGCGGCGTGCTCTTAAAC
	AAATGCATTTATTCACGAAGCAGTGGATGTG
pLG5186	CACTAACTTCATGATGACCGAATACGTGGTGACACGGTAC
	GTACCGTGTCACCACGTATTCGGTCATCATGAAGTTAGTG
pSK4611	AGCGTCGACATGCGTCCTACATCCCTTAAAC
	CAGCATGCTAGCTAAAGTAATGGATGCAG
pKB4505	AGAATTCCTTAATGCGCCCTACATCCC
	ACCGTCGACCAACGTAGCTAAAGTAACGG
pKB4506	AGAATTCATGGTATTTTCAGCCAAACC
	ACCGTCGACCAACGTAGCTAAAGTAACGG
pKB4699	CAGGGATCCATGCTTGACTTTGGCCTGGCCCGGACAGCGTG CACTAACTTCATGATGACCCCTTACGTGGTGACACGGTACT ACCGGATGGTGAGCAAGGGCGAG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pLG6728	ATGTCAGATACAGATATCGAGTCTCTTGTAAAAG
	GCCCTGAAAATACAGGTTTTCGTGATGATGATGATGATGGC TGC

pLG6725	TTCATGATGACACCATATGTGGTCACACGTTATTACAGAGC TCCAGAAATTATGTTGAATTCC
	ATTTGTACATGCGGTACGGGCCAGGCCAAAGTCCAAGATCT TGAGATCACAAGTGGTGTTCAG
pLG6726	CATACAGACGACGAAATGACAGGATATGTAGCCACGCGTT GGTACAGAG
	ACGGGCAAGGCCAAAGTCCAAGATCTTGAGATCACAAGTG GTGTTCAG
pLG6727	GATCCAGACCATGATCATACAGGGTTCTTGACAGAGTACGT GGCCACTAGGTGGTACAGG
	TGCAACCCGAGCCAGTCCAAAATCACAAATCTTCAGCTCAC AGTCTTCATTCACAG
pLG6680	GTATGCCCCCGCCGAAGCAGAAGC
	CGGGGGCATACCAAACAGTGATGTATGGG
pLG6681	CGCCGCAGCAGAAGCCCCACCACC
	CTGCTGCGGCGGGGTCATACCAAAC
pLG6682	AGCAGCAGCCCCACCACCTCAAATTTATG
	GGGCTGCTGCTTCGGCGGGGTCATAC
pLG6683	CCCCCGCCGCAGCAGCAGCCCCACCACCTCAAATTTATG
	GCTGCTGCGGCGGGGGCATACCAAACAGTGATGTATGGGT G

References

Baruch, K., Gur-Arie, L., Nadler, C., Koby, S., Yerushalmi, G., Ben-Neriah, Y., Yogev, O., Shaulian, E., Guttman, C., Zarivach, R.*, et al.* (2011). Metalloprotease type III effectors that specifically cleave JNK and NF-kappaB. The EMBO journal *30*, 221-231.

Schlosser-Silverman, E., Elgrably-Weiss, M., Rosenshine, I., Kohen, R., and Altuvia, S. (2000). Characterization of Escherichia coli DNA lesions generated within J774 macrophages. Journal of bacteriology *182*, 5225-5230.

Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Soding, J.*, et al.* (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular systems biology *7*, 539.