

Supporting Information

Selective MAP kinases proteolysis determined by activation loop flexibility

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Hamiltonella defensa (HD)	MR-RALKLNLTPLSFSS-----SSNSDTDIQSLVKKSSVNWIKNNHQLSFDGTNKN
Erwinia_piriflorinigrans	MRPTSLQLTQPVNLPLPSS-----DPASDIQSLVKISGVNWNINNNQQLSFIGDTYKN
Shigella_boydii	MRPTSLNLALHQSS-----TSSMSDADIESLVKTSVQWIKNNPQLRFQGTDDHNI
Enterohemorrhagic E.coli (EHEC)	MRPTSLNLVHLHQSS-----RSSMSDTDIESLVKASSVQWIKNNPQLRFQGTDDHNI
Enteropathogenic E.coli (EPEC)	MRPTSLNLVHLHQSS-----TSSMSDTDIESLVKASSVQWIKNNPQLRFQGTDDHNI
Escherichia_albertii	MRPTSLNLALHQSS-----TSSMSDTDIESLVKASSVQWIKNNPQLRFQGTDDHNI
Providencia_alcalifaciens	MRPTSLKLTLPSPSTSSS-----HSSSATDIQYLVKMSVSWIKNNQQLHFLGTDHKI
Izhakiella_capsodis	MRPTFLNLTSPSSSLPFSNSNSNSNSTSTTDIQSLVKMSRVHWVKNQQLCFHGTDHKI
Salmonella_enterica_arizonae (SEA)	MRPTSLKLTLPSPSTSSS-----NS--ATDIQSLVKRSGVRWIKNNQQLSFIGTDHKI
Pantoea_stewartii	MRPTSLNLVHLPS--HLPSSS-----NSISATDVQSLVKMSSVLWIKNNQQLCFHGTDHKI
Salmonella_enterica_Sandiego	MRPTSLKLTLPSPSTSSS-----NSISATDIQSLVKRSGVRWVKNQQLSFIGTDYKI
Leclercia	MRPTSLNLTLPSLPSPSS-----NSISATDIQSHVKMSGVHWVKNQQLCFHGTDLKI
Salmonella_enterica_Braenderup	MRPTSLNLTLPSLPSPSS-----NSVSATDIQSLVKMSGVRWVKNQQLCFHGADLKI
Salmonella_enterica_salamae	MRPTSLNLTLPSLPSPSS-----NSVSATDIQSLVKMSGVRWVKNQQLCFHGADLKI
Citrobacter_rodentium (CR)	MRPTSLNLTLPSLPSPSS-----NSISATDIQSLVKMSGVRWVKNQQLCFHGTDLKI
Erwinia_tracheiphila	MRPTSLNLTLPSLPSPSS-----NSISATDIQSLVKISGVVWVKNQQLCFHGTDLKI
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Hamiltonella defensa (HD)	YQRLEKALDKIESTETGKELLNCIESVSRKSEKLIIRLDSTELGVTAHCAENAENFRGT
Erwinia_piriflorinigrans	YRQLETALDKVKSNTGRTLLKCIALTSQLKSKLAIIFLNSEELMVEPHCKTDAENFRGT
Shigella_boydii	YQQTAAALDKIGSTETGRVLLNAIESISRLKSETVVIHLSNSSLGVIHAHRDIDAENHRGT
Enterohemorrhagic E.coli (EHEC)	YQQTAAALDKIGSTETGRVLLNAIESISRLKSETVVIHLSNSSLGVIHAHRDIDAENHRGT
Enteropathogenic E.coli (EPEC)	YQQTAAALDKIGSTETGRVLLNAIESISRLKSETVVIHLSNSSLGVIHAHRDIDAENHRGT
Escherichia_albertii	YQQTAAALDKIGSTETGRVLLNAIESISRLKSETVVIHLSNSSLGVIHAHRDIDAENHRGT
Providencia_alcalifaciens	YQKLEAALDKIESTNTGRILLNSELTSRLKSEKLVIIHLSSELGVIHAHCNTDAENSRGT
Izhakiella_capsodis	YQHLEAALDKIESTDTGRTLLNCIELTSRLKSEKLAIIYLNCTELMVAHCADDAENSRGT
Salmonella_enterica_arizonae (SEA)	YQQLAALDKIESTDTGRTLLKCIELTSQLKSEKLAIIHLSAELGVVAHCNTDAENARGT
Pantoea_stewartii	YQQLAALDKIESTNTGRTLLNGIGLTSRLKSEKLAIIHLSAELGVVAHCNTDAENFRGT
Salmonella_enterica_Sandiego	YQQLAALDKIESTDTGRTLLNCIELTSRLKSEKLEIHLNSAELGVVAHCNTDAENSRGT
Leclercia	YQHLEAALDKIESTDTGRTLLNCIELTSRLKSEKLAIIHLSAELGVVAHCNTDAENSRGT
Salmonella_enterica_Braenderup	YQHLEAALDKIESTDTGRTLLNCIELTSRLKSEKLAIIHLSAELGVVAHCNTDAENSRGT
Salmonella_enterica_salamae	YQHLEAALDKIESTDTGRTLLNCIELTSRLKSEKLAIIHLSAELGVVAHCNTDAENSRGT
Citrobacter_rodentium (CR)	YQHLEAALDKIESTDTGRTLLNCIELTSRLKSEKLAIIHLSAELGVVAHCNTDAENSRGT
Erwinia_tracheiphila	YKHLAALDKIESTDTGRTLLNCIELTSRLKSEKLAIIHLSAELGVVAHCNTDAENSRGT
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Hamiltonella defensa (HD)	GSYLHCLNLSVQEEESIGKIGQTELDACIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Erwinia_piriflorinigrans	GSDDFHCNFDVAE-YFFDQGMDLVEFQACVVEHELLHVFNHNLNGERLKVAVFQPEVETYS
Shigella_boydii	GSDDFHCNNAVE-YPCGEGISVVDVFHATIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Enterohemorrhagic E.coli (EHEC)	GSDDFHCNNAVE-YPCGEGISVVDVFHATIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Enteropathogenic E.coli (EPEC)	GSDDFHCNNAVE-YPCGEGISVVDVFHATIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Escherichia_albertii	GSDDFHCNNAVE-YPCGEGISVVDVFHATIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Providencia_alcalifaciens	GSDDFHCNNAVE-YPCGQGISLVDVFHACIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Izhakiella_capsodis	GSNFHCNNAVE-YPCGQGISLVDVFHACIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Salmonella_enterica_arizonae (SEA)	GSDDFHCNNAVE-YPCGQGISLVDVFHACIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Pantoea_stewartii	GSDDFHCNNAVE-YPCGQGISLVDVFHACIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Salmonella_enterica_Sandiego	GSDDFHCNNAVE-YPCGQGISLVDVFHACIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Leclercia	GSDDFHCNNAVE-YPCGQGISLVDVFHACIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Salmonella_enterica_Braenderup	GSDDFHCNNAVE-YPCGQGISLVDVFHACIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Salmonella_enterica_salamae	GSDDFHCNNAVE-YPCGQGISLVDVFHACIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Citrobacter_rodentium (CR)	GSDDFHCNNAVE-YPCGQGISLVDVFHACIVHELLHVFNHNLNGERLKVAVFQPEVETYS
Erwinia_tracheiphila	GSDDFHCNNAVE-YPCQGISLVDVFHACIVHELLHVFNHNLNGERLKVAVFQPEVETYS
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Hamiltonella defensa (HD)	FLLEEARTVGLGSFSEEVLSENKFREEIGIPRRVSYAHESLIHDDNTFTMFFENKQSHPLL
Erwinia_piriflorinigrans	LLLEEARTVGLGFFSSEYLSENKFRKEIGAPRRTSYPYDFYIVNDNTITMGYERKKLHPLL
Shigella_boydii	LLLEEARTVGLGAFSEEVLSENKFREEIGMPRRTSYPHDSALIHDDNTVSLGQVRLHPLL
Enterohemorrhagic E.coli (EHEC)	LLLEEARTVGLGAFSEEVLSENKFREEIGMPRRTSYPHDSALIHDDNTVSLGQVRLHPLL
Enteropathogenic E.coli (EPEC)	LLLEEARTVGLGAFSEEVLSENKFREEIGMPRRTSYPHDSALIHDDNTVSLGQVRLHPLL
Escherichia_albertii	LLLEEARTVGLGAFSEEVLSENKFREEIGMPRRTSYPHDSALIHDDNTVSLGQVRLHPLL
Providencia_alcalifaciens	LLLEEARTVGLGAFSEEVLSENKFREEIGIPRRTSYPHDSALIHDDNTVSLGQVRLHPLL
Izhakiella_capsodis	LLLEEARTVGLGAFSEEVLSENKFRVEIGIPRRTFYSHDLAIHDDNTVTQGFQRRKHLHPLL
Salmonella_enterica_arizonae (SEA)	FLLEEARTVGLGSFSEEVLSENKFREEIGVPRRTFYPRDLYIHDDNTVTQGLQRKHLHPLL
Pantoea_stewartii	LLLEEARTVGLGAFSEEVLSENKFREEIGVPRRTFYPHDSSLIHDDNTVTQGFQRRKHLHPLL
Salmonella_enterica_Sandiego	FLLEEARTVGLGSFSEEVLSENKFREEIGVPRRTFYPHNSLIHDDNTVVSQGFQRRKHLHPLL
Leclercia	LLLEEARTVWLGAFSEEVLSENKFREEIGMPRRTFYPRDSSLIHDDNTVTQGFQRRKHLHPLL
Salmonella_enterica_Braenderup	LLLEEARTVGLGAFSEEVLSKFREEIGMPRRTFYPHDSSLIHDDNTVTQGFQRRKHLHPLL
Salmonella_enterica_salamae	LLLEEARTVGLGAFSEEVLSENKFREEIGMPRRTFYPHDSSLIHDDNTVTQGFQRRKHLHPLL
Citrobacter_rodentium (CR)	LLLEEARTVGLGAFSEEVLSENKFREEIGMPRRTFYPHDSSLIHDDNTVTQGFQRRKHLHPLL
Erwinia_tracheiphila	LLLEEARTVGLGAFSEEVLSENKFREEIGMPRRTFYPHDSSLIHDDNTVTQGFQRRKHLHPLL
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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.

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 $\Delta nleD::kan$	(Baruch et al., 2011)
BL21(DE3)	F-, <i>ompT</i> , <i>hsdS</i> β ($r\beta$ - $m\beta$ -), <i>dcm</i> , <i>gal</i> , (DE3) <i>tonA</i>	Novagen
KB4583	<i>Salmonella enterica</i> serovar Arizona (SEA) strain CDC346-86 SARC5	ATCC
ICC168 (4368)	<i>Citrobacter rodentium</i>	Gad Frankel
Rosetta	<i>E. coli</i> 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
p38 α	pET28a, bacterial expression vector encoding hexa-His tag p38 α	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 <i>nleD</i> _{EPEC 19-232}	This study	pLG6728
ERK _A -JNK	pET15b, bacterial expression vector encoding ERK2 with JNK2 activation loop (residues 169-188 from JNK2)	This study	pLG6725
ERK _A -p38	pET15b, bacterial expression vector encoding ERK2 with p38 α activation loop (residues 167-185 from p38 α)	This study	pLG6726
p38 _A -ERK	pET28a, bacterial expression vector encoding p38 α with ERK2 activation loop (residues 164-188 from ERK2)	This study	pLG6727
ERK _G -p38	pET15b, bacterial expression vector encoding ERK2 with G-helix from p38 α (residues 223-229 from p38 α)	This study	
ERK _G -JNK	pET15b, bacterial expression vector encoding ERK2 with G-helix from JNK2 (residues 225-231 from JNK2)	This study	
ERK _{G,M,L} -p38	pET15b, bacterial expression vector encoding ERK2 with G-helix, MKI and L16 from p38 α (residues 223-229, 243-262, 327-333, respectively, from p38 α)	This study	
ERK _{G,M,L} -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 -p38	pET15b, bacterial expression vector encoding ERK2 with α EF/ α F loop from p38 α (residues 196-202 from p38 α)	This study	
ERK _{G,F} -p38	pET15b, bacterial expression vector encoding ERK2 with α EF/ α F loop and G-helix from p38 α (residues	This study	

	196-202 and 223-229, respectively, from p38 α)		
ERK _{G,F,M,L-p38}	pET15b, bacterial expression vector encoding ERK2 containing ERtrp38+ ER α EF/ α Fp38	This study	
p38 α D316N	pET28a, bacterial expression vector encoding hexa-His tag p38 α with D316N	Lab collection	
pET28a	Bacterial expression vector	Novagen	pLG4483
NleD EPEC	pET28a, bacterial expression vector encoding <i>nleD</i> _{EPEC}	This study	pLG4480
pSA10	pKK177-3 derivative containing <i>lacI</i> ^q . bacterial expression vector.	(Schlosser-Silverman et al., 2000)	1060
NleD EPEC	pSA10, bacterial expression vector encoding <i>nleD</i> _{EPEC}	(Baruch et al., 2011)	pKB4345
NleD CR1	pSA10, bacterial expression vector encoding <i>nleD1</i> _{CR}	This study	pKB4505
NleD CR2	pSA10, bacterial expression vector encoding <i>nleD2</i> _{CR}	This study	pKB4506
NleD SEA	pSA10, bacterial expression vector encoding <i>nleD</i> _{SEA}	This study	pSK4611
NleD HD	pSA10, bacterial expression vector encoding <i>nleD</i> _{HD}	This study	pSK4702
GST-7Ala-GFP	pGEX2T, bacterial expression vector encoding GST – 7 alanine loop – GFP	This study	pLG5088
GST- JNK ₁₆₉₋₁₉₂ -GFP	pGEX2T, bacterial expression vector encoding GST –JNK2 _{D169 – R192} – GFP	This study	pKB4699
GST- JNK ₁₇₆₋₁₉₂ -GFP	pGEX2T, bacterial expression vector encoding GST –JNK2 _{A176 – R192} – GFP	This study	pLG5011
GST- JNK ₁₈₀₋₁₉₂ -GFP	pGEX2T, bacterial expression vector encoding GST –JNK2 _{F180 – R192} – GFP	This study	pLG5102
GST- JNK ₁₇₆₋₁₈₈ -GFP	pGEX2T, bacterial expression vector encoding GST –JNK2 _{A176 – T188} – GFP	This study	pLG5101
GST- JNK ₁₈₀₋₁₈₆ -GFP	pGEX2T, bacterial expression vector encoding GST –JNK2 _{F180 – V186} – GFP	This study	pLG5086
GST- JNK ₁₈₂₋₁₈₈ -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 ₁₈₃₋₂₁₇ – 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
p38 β	pET28a, bacterial expression vector encoding hexa-His tag p38 β	David Engelberg	pLG4495
ERK1	pET28a, bacterial expression vector encoding hexa-His tag ERK1	This study	pLG4774
JNK1	pSR α 3, mammalian expression vector encoding HA-JNK1,	Eitan Shaulian	pLG4487
JNK2	pSR α 3, mammalian expression vector encoding HA-JNK2	Eitan Shaulian	pLG4488
p38 α	pcDNA3, mammalian expression vector encoding HA-p38 α	David Engelberg	pLG4683
p38 β	pcDNA3, mammalian expression vector encoding HA-p38 β	David Engelberg	pLG4684
p38 γ	pcDNA3, mammalian expression vector encoding HA-p38 γ	David Engelberg	pLG4685
p38 δ	pcDNA3, mammalian expression vector encoding HA-p38 δ	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	pSR α 3, mammalian expression vector encoding HA-JNK2 with D326A mutation	This study	pLG6680
JNK2 E329A	pSR α 3, mammalian expression vector encoding HA-JNK2 with E329A mutation	This study	pLG6681
JNK2 E331A	pSR α 3, mammalian expression vector encoding HA-JNK2 with E331A mutation	This study	pLG6682
JNK2 D326A/ E329A/ E331A	pSR α 3, mammalian expression vector encoding HA-JNK2 with 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	CAGGGATCCGCGTGCACCTAATTCATGATGACCCCTTACGT
	GGTGACACGGTACTACCGGATGGTGAGCAAGGGCGAG
pLG5086	CCAGAATTCTTACTTGTACAGCTCGTCCATG
	CAGGGATCCTTCATGATGACCCCTTACGTGATGGTGAGCAA
pLG5087	GGGCGAG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pLG5088	CAGGGATCCATGACCCCTTACGTGGTGACAATGGTGAGCAA
	GGGCGAG
pLG5101	CCAGAATTCTTACTTGTACAGCTCGTCCATG
	CAGGGATCCGCGTGCACCTAATTCATGATGACCCCTTACGT
pLG5102	GGTGACAATGGTGAGCAAGGGCGAG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pLG5103	CAGGGATCCTTCATGATGACCCCTTACGTGGTGACACGGTA
	CTACCGGATGGTGAGCAAGGGCGAG
pLG5103	CCAGAATTCTTACTTGTACAGCTCGTCCATG
	CAGGGATCCTGTGATTTGCGCCTGGCCCGGATTGCCGATCC
pSK4702	TGAGCATGACCACACCGGCTTCTGACGGAGTATGTGGCTA
	CGCGCTGGATGGTGAGCAAGGGCGAG
pLG5186	CCAGAATTCTTACTTGTACAGCTCGTCCATG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pSK4611	ATCGGACCCGGGATGCGGCGTGCTCTTAAAC
	AAATGCATTTATTCACGAAGCAGTGGATGTG
pKB4505	CACTAATTCATGATGACCGAATACGTGGTGACACGGTAC
	GTACCGTGTCACCACGTATTCGGTCATCATGAAGTTAGTG
pKB4506	AGCGTCGACATGCGTCCTACATCCCTTAAAC
	CAGCATGCTAGCTAAAGTAATGGATGCAG
pKB4699	AGAATTCCTTAATGCGCCCTACATCCC
	ACCGTCGACCAACGTAGCTAAAGTAACGG
pLG6728	AGAATTCATGGTATTTTCAGCCAAACC
	ACCGTCGACCAACGTAGCTAAAGTAACGG
pKB4699	CAGGGATCCATGCTTGACTTTGGCCTGGCCCGGACAGCGTG
	CACTAATTCATGATGACCCCTTACGTGGTGACACGGTACT
pLG6728	ACCGGATGGTGAGCAAGGGCGAG
	CCAGAATTCTTACTTGTACAGCTCGTCCATG
pLG6728	ATGTCAGATACAGATATCGAGTCTCTTGTA AAAAG
	GCCCTGAAAATACAGGTTTTTCGTGATGATGATGATGATGGC
	TGC

pLG6725	TTCATGATGACACCATATGTGGTCACACGTTATTACAGAGC TCCAGAAATTATGTTGAATTCC
	ATTTGTACATGCGGTACGGGCCAGGCCAAAGTCCAAGATCT TGAGATCACAAGTGGTGTTCAG
pLG6726	CATACAGACGACGAAATGACAGGATATGTAGCCACGCGTT GGTACAGAG
	ACGGGCAAGGCCAAAGTCCAAGATCTTGAGATCACAAGTG GTGTTCAG
pLG6727	GATCCAGACCATGATCATACAGGGTTCTTGACAGAGTACGT GGCCACTAGGTGGTACAGG
	TGCAACCCGAGCCAGTCCAAAATCACAAATCTTCAGCTCAC AGTCTTCATTACAG
pLG6680	GTATGCCCCCGCCGAAGCAGAAGC
	CGGGGGCATAACAAACAGTGATGTATGGG
pLG6681	CGCCGCAGCAGAAGCCCCACCACC
	CTGCTGCGGCGGGGTCATAACAAAC
pLG6682	AGCAGCAGCCCCACCACCTCAAATTTATG
	GGGCTGCTGCTTCGGCGGGGTCATAC
pLG6683	CCCCCGCCGCAGCAGCAGCCCCACCACCTCAAATTTATG
	GCTGCTGCGGCGGGGGCATAACAAACAGTGATGTATGGGT G

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