# SUPPLEMETARY INFORMATION

## Supplementary Table 1: List of primers used in this study

| Primer Name | Sequence |
|---|---|
| **DhNIK1 cloning** | |
| Dnik1F-BamH1 | 5’ CCGGATCCGTGTCGCATACTCATCC 3’ |
| DnikP-Nco1R | 5’ CATGCCATGGTCAACGCTTGAATCAA 3’ |
| Dnik-Nco1F | 5’ CATGCCATGGGTACACCCGAATTAATGAA 3’ |
| Dnik-Xho1R | 5’ CCGCTCGAGTCAATCAAGATATTCAATCCTTGG 3’ |
| DnikR Xho1-GFP | 5’ CCGCTCGAGAATCAAGATATTCAATCCTTGGA 3’ |
| **H500Q mutation** | |
| DnikH500QF1 | 5’CAAATATGTCCCAAGAGATTAGGACACCTT 3’ |
| DnikH500QR1 | 5’ TCCTAATCTCTTGGGACATATTTGCCAAAA 3’ |
| HAMPR-EcoR1 | 5’ ACAGAATTCCAATAATAACCTTTCCTTG 3’ |
| **D915R mutation** | |
| Dnik D915N F1 | 5’ GTTTTAATGAATGTACAAATGCCTGTAATG 3’ |
| Dnik D915N R1 | 5’ GCATTTGTACATTCATTAAAACAACATCGT 3’ |
| DNik1 F 2 -EcoR1 | 5’TTGGAATTCTGTGTCAGTGATACT 3’ |
| **H13452 construct** | |
| 1CR | 5’ CTTCGATACTTCGTTTGCAAATGCTTGCAA3’ |
| 1CF | 5’ GCATTTGCAAACGAAGTATCGAAGGTTGCT3’ |
| 5BR | 5’ TTTTGTCACTTCTTGAATAGATTCTCTTAA3’ |
| 5BF | 5’ GAATCTATTCAAGAAGTGACAAAAGTTGCG3’ |
| 2fR | 5’AGCGGTGTTTCTAAAGGCAAATGTTCTTAA3’ |
| 2fF | 5’ACATTTGCCTTTAGAAACACCGCTGCAAGG3’ |
| **H12435 construct** | |
| C4 rev | 5’AGTTAAATTAGTAGCCATAGCATTTACATT3’ |
| C4 for | 5’AATGCTATGGCTACTAATTTAACTAATCAA3’ |
| E3 rev | 5’CGTCAAATTAAGAGCCATAGTGTTGACATT3’ |
| E3for | 5’AACACTATGGCTCTTAATTTGACGACCCAA3’ |
| D5rev | 5’CGTTAAATTAGAAGCCATAAGATTAACATT3’ |
| D5for | 5’AATCTTATGGCTTCTAATTTAACGTCGCAA3’ |
| **Individual HAMP domain deletion** | |

| | |
|---|---|
| ΔH1delF | 5’ TCGGCCAATAAGGAAGTGACAAAAGTTGCGACTGAA 3’ |
| ΔH1delR | 5’ TTTTGTCACTTCCTTATTGGCCGACTTGGAGGTATC 3’ |
| ΔH2delF | 5’ AACATTATGGCCGAAGTATCGAAGGTTGCTAGAGAT 3’ |
| ΔH2delR | 5’ CTTCGATACTTCGGCCATAATGTTAACATTATCAGT 3’ |
| ΔH3delF | 5’ AATGCTATGGCTGAAGTTACTACATTATCCCGTGAA 3’ |
| ΔH3delR | 5’ TGTAGTAACTTCAGCCATAGCATTTACATTATCTGT 3’ |
| ΔH4delF | 5’ AATCTTATGGCTGAAGTGTCAAAGGTCGCAAGAGAT 3’ |
| ΔH4delR | 5’ CTTTGACACTTCAGCCATAAGATTAACATTCTCTGT 3’ |
| ΔH5delF | 5’ AACACTATGGCTAGAAACACCGCTGCAAGGGAAGCT 3’ |
| ΔH5delR | 5’ AGCGGTGTTTCTAGCCATAGTGTTGACATTACTGGT 3’ |

**For serial HAMP domain deletions**

| | |
|---|---|
| ΔH1-2delF | 5’ TCGGCCAATAAGGAAGTATCGAAGGTTGCTAGAGAT 3’ |
| ΔH1-2delR | 5’ CTTCGATACTTCCTTATTGGCCGACTTGGAGGTATC 3’ |
| ΔH1-3delF | 5’ TCGGCCAATAAGGAAGTTACTACATTATCCCGTGAA 3’ |
| ΔH1-3delR | 5’ TGTAGTAACTTCCTTATTGGCCGACTTGGAGGTATC 3’ |
| ΔH1-4delF | 5’ TCGGCCAATAAGGAAGTGTCAAAGGTCGCAAGAGAT 3’ |
| ΔH1-4delR | 5’ CTTTGACACTTCCTTATTGGCCGACTTGGAGGTATC 3’ |
| ΔH1-5delF | 5’ TCGGCCAATAAGAGAAACACCGCTGCAAGGGAAGCT 3’ |
| ΔH1-5delR | 5’ AGCGGTGTTTCTCTTATTGGCCGACTTGGAGGTATC 3’ |

**Two hybrid cloning**

| | |
|---|---|
| HAMP1_twF | 5' CGGAATTCATGGGTACACCCGAATTAA 3' |
| HAMP1_twR | 5' CCGCTCGAGTTAGTTTGCAAATGCTTGCAA 3' |
| HAMP2_twF | 5' CGGAATTCGAAGTGACAAAAGTTGCGA 3' |
| HAMP2_twR | 5' CCGCTCGAGTTAAAAGGCAAATGTTCTTAACT 3' |
| HAMP3_twF | 5' CGGAATTCGAAGTATCGAAGGTTGCTAG 3' |
| HAMP3_twR | 5' CCGCTCGAGTTACAATGCAAAATTCTGTAATC 3' |
| HAMP4_twF | 5' CGGAATTCGAAGTTACTACATTATCCCG 3' |
| HAMP4_twR | 5' CCGCTCGAGTTATGATGCGAAAAGTTGTAAA 3' |
| HAMP5_twF | 5' CGGAATTCGAAGTGTCAAAGGTCGCA 3' |
| HAMP5_twR | 5' CCGCTCGAGTTATTGAATAGATTCTCTTAAATTCA 3' |

**HA-tagging**

DNIK1_ORF_R_2HA
5’CCGCTCGAGTTATGCATAGTCCGGGACGTCATACGGATAGCCCGCATAGTCAGGAACATCGTATGGGTAATCAAGATATTCAATCCTTGGAGAA 3’

**His-tagged protein**

| | |
|---|---|
| DNik1R-XhoI | 5’CCGCTCGAGTGCATCAAGATATTCAATCCTT 3’ |

## Supplementary Table 2: Amino acid sequences of the individual HAMP domains from *NIK1* orthologs from different yeast and fungi

| Name | Sequence |
|---|---|
| *Yarrowia lipolytica* | |
| YL5H1 | RAFQKALMEIGEVVFAVAQGDLSKKVTIHDQEMDPEIVKFKETINKMMSQLQRFAS |
| YL5H2 | SNLTNQVREIADVTRAVAQGDLSRKINVHAQGEILELQKTINTMVEQLRTFAF |
| YL5H3 | SNLTDQVRNIANVTTAVARGDLSQKVSADCKGEILDLKSTINKMVDRLQNFAL |
| YL5H4 | SNLTTQVRSIAHVTTAVAQGDLSQKIDVHAQGEMLALKSTINKMVDRLQVFAS |
| YL5H5 | ANLTTQVRALVQITAAATDGDFTRFITVEASGEMDALKTKINQMVLNLRESIQ |
| *Lodderomyces elongisporus* | |
| LE5H1 | KAFKQALSEIGTVVISVAMGDLSKKVEIHTVESDPEILKVKITINTMMDQLQTFAS |
| LE5H2 | SNLTNQVREIADVTRAVARGDLSRKINVHAQGEILQLQMTINTMVDQLRTFAF |
| LE5H3 | LNLTTQVRNIANVTTAVAKGDLSKKVTADCKGEILNLKLTINQMVDRLLKFAS |
| LE5H4 | TNLTNQVRSIATVTTAVAHGDLSQKIDVHAQGEILQLKNTINKMVDSLQLFAS |
| LE5H5 | SNLTSQVRAFAQITAAATDGDFTRFITVEALGEMDALKTKINQMVLNLRESIQ |
| *Pichia stipitis* | |
| PS5H1 | KAFKQALSEIGTVVISVAMGDLSKKVEIHTVESDPEILKVKITINTMMDQLQAFAN |
| PS5H2 | LNLTNQVREIADVTRAVARGDLSRKINVHAQGEILQLQMTINTMVDQLRTFAF |
| PS5H3 | LNLTTQVRNIANVTTAVAKGDLSKKVTADCKGEILNLKLTINQMVDRLQNFAL |
| PS5H4 | TNLTNQVRSIATVTTAVAHGDLSQKIDVHAQGEILQLKNTINKMVDSLQLFAS |
| PS5H5 | SNLTSQVRAFAQITAAATDGDFTRFITVEASGEMDALKTKINQMVLNLRESIQ |
| *Debaryomyces hansenii* | |
| DH5H1 | KAFKQALSEIGTVVISVAMGDLSKKVEIHTVESDPEILKVKITINTMMDQLQAFAN |
| DH5H2 | LNLTNQVREIADVTRAVARGDLSRKINVHAQGEILQLQMTINSMVDQLRTFAF |
| DH5H3 | LNLTTQVRNIANVTTAVAKGDLSKKVTADCKGEILDLKLTINQMVDRLQNFAL |
| DH5H4 | TNLTNQVRSIATVTTAVAHGDLSQKIDVHAQGEILQLKNTINKMVDSLQLFAS |
| DH5H5 | SNLTSQVRAFAQITAAATDGDFTRFITVEASGEMDALKTKINQMVLNLRESIQ |
| *Candida albicans* | |
| CA5H1 | KAFQQALSEIGTVVISVAMGDLSKKVEIHTVENDPEILKVKITINTMMDQLQTFAN |
| CA5H2 | LNLTNQVREIADVTRAVAKGDLSRKINVHAQGEILQLQRTINTMVDQLRTFAF |
| CA5H3 | LNLTTQVRNIANVTTAVAKGDLSKKVTADCKGEILDLKLTINQMVDRLQNFAL |
| CA5H4 | TNLTNQVRSIATVTTAVAHGDLSQKIDVHAQGEILQLKNTINKMVDSLQLFAS |
| CA5H5 | SNLTSQVRAFAQITAAATDGDFTRFITVEASGEMDALKTKINQMVFNLRESLQ |
| *Botryotinia fuckeliana* | |
| BF6H1 | EAFQKALREIGEIVTAVARGDLSKKVQIHSVEMDPEITTFKRVINTMMDQLQIFSS |
| BF6H2 | QNLTDQVREIASVTTAVAHGDLTQKIERPAQGEILQLQQTINTMVDQLRTFAA |
| BF6H3 | NNLTTQVRDIAIVTTAVAKGDLTQKVQAECKGEIKQLKETINSMVDQLQQFAR |
| BF6H4 | MNLTTQVREIAKVTTAVARGDLTKKIEVEVQGEIASLKDTINTMVDRLSTFAF |
| BF6H5 | RNLTTQVRGISTVTQAIANGDMSQKIEVAAAGEILILKETINNMVDRLSIFSN |
| BF6H6 | NNLTTQVRAFGDITNAATDGDFTKLITVEASGEMDELKRKINQMVYNLRDSIQ |
| *Gibberella moniliformis* | |
| GM6H1 | EAFQKALREIGEIVTAVARGDLTMKVRMNTVEMDPEITTFKRTINAMMDQLQIFAS |
| GM6H2 | KELTDNVREIASVTTAVAHGDLTKKIERPARGEILQLQQTINTMVDQLRTFAS |

| | |
|---|---|
| GM6H3 | NNLTTQVRDIIKVTTAVAKGDLTQKVQADCRGEIFELKSTINSMVDQLQQFAR |
| GM6H4 | MNLTTQVREIAKVTTAVAKGDLTKKIGVEVKGEIAELKNTINQMVDRLGTFAV |
| GM6H5 | SNLTVQVRSISTVTQAIANGDMSQKIKVEANGEIQVLKETINNMVDRLSSFCY |
| GM6H6 | SNLTTQVRAFSDITNLATDGDFTKLVDVEASGEMDELKRKINQMISNLRDSIQ |

*Neurospora crassa*

| | |
|---|---|
| NC6H1 | EAFQKALREIGEIVTAVARGDLSKKVRMNSVEMDPEITTFKRTINTMMDQLQVFSS |
| NC6H2 | QNLTDQVREIASVTTAVAHGDLTKKIERPAKGEILQLQQTINTMVDQLRTFAS |
| NC6H3 | NNLTTQVRDIIKVTTAVAKGDLTQKVQAECRGEIFELKKTINSMVDQLQQFAR |
| NC6H4 | MNLTTQVREIAKVTTAVAKGDLTKKIGVEVQGEILDLKNTINTMVDRLGTFAF |
| NC6H5 | SNLTSQVRGISTVTQAIANGDMSRKIEVEAKGEILILKETINNMVDRLSIFCN |
| NC6H6 | NNLTAQVRAFGDITNAATDGDFTKLVEVEASGEMDELKKKINQMVYNLRDSIQ |

*Magnaporthe grisea*

| | |
|---|---|
| MG6H1 | EAFQKALREIGSIVTAAARGDLSKRVKINPIEMDPEITTFKRTMNAMMDQLGVFSS |
| MG6H2 | QNLTDQVREIASVTTAVAHGDLTQKIESAAKGEILQLQQTINTMVDQLRTFAS |
| MG6H3 | NNLTTQVRDIINVTTAVAKGDLTQKVQAECRGEIFELKNTINSMVDQLQQFAR |
| MG6H4 | MNLTTQVREIANVTSAVAAGDLSKKIRVEVKGEILDLKNTINTMVDRLGTFAF |
| MG6H5 | SNLTSQVRGISTVTQAIANGDMSRKIDVEAKGEILILKETINNMVDRLSIFCN |
| MG6H6 | NNLTAQVRAFGDITNAATDGDFTKLVEVEASGEMDELKRKINQMVYNLRDSIQ |

*Aspergillus niger*

| | |
|---|---|
| AN6H1 | EAFQKALREIGGIITQVANGDLSMKVQIHPLEMDPEIATFKRTINTMMDQLQVFGS |
| AN6H2 | KNLTDQVREIAAVTTAVAHGDLSQKIESRAQGEILELQQTINTMVDQLRTFAT |
| AN6H3 | NNLTTQVRDIATVTKAVAKGDLTQKVQANCKGEIAELKNIINSMVDQLRQFAQ |
| AN6H4 | NNLTTQVREIADVTTAVAKGDLTKKVTANVQGEILDLKSTINGMVDRLNTFAF |
| AN6H5 | QNLTSQVRSISDVTQAIAKGDLSKKIEVHAQGEILTLKVTINHMVDRLAKFAT |
| AN6H6 | ENLTSQVRAFGEITDAATDGDFTKLITVNASGEMDELKRKINKMVSNLRDSIQ |

## Supplementary Figure 1: CLUSTAL X sequence alignment of HAMP domains

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BF6H1          EAFQKALREIGEIVTAVARGDLSKKVQIHSVEMDPEITTFKRVINTMMDQLQIFSS
NC6H1          EAFQKALREIGEIVTAVARGDLSKKVRMNSVEMDPEITTFKRTINTMMDQLQVFSS
MG6H1          EAFQKALREIGSIVTAAARGDLSKRVKINPIEMDPEITTFKRTMNAMMDQLGVFSS
GM6H1          EAFQKALREIGEIVTAVARGDLTMKVRMNTVEMDPEITTFKRTINAMMDQLQIFAS
AN6H1          EAFQKALREIGGIITQVANGDLSMKVQIHPLEMDPEIATFKRTINTMMDQLQVFGS
YL5H1          RAFQKALMEIGEVVFAVAQGDLSKKVTIHDQEMDPEIVKFKETINKMMSQLQRFAS
PS5H1          KAFKQALSEIGTVVISVAMGDLSKKVEIHTVESDPEILKVKITINTMMDQLQAFAN
DH5H1          KAFKQALSEIGTVVISVAMGDLSKKVEIHTVESDPEILKVKITINTMMDQLQAFAN
LE5H1          KAFKQALSEIGTVVISVAMGDLSKKVEIHTVESDPEILKVKITINTMMDQLQTFAS
CA5H1          KAFQQALSEIGTVVISVAMGDLSKKVEIHTVENDPEILKVKITINTMMDQLQTFAN
LE5H4          TNLTNQVRSIATVTTAVAHGDLSQKIDVHAQG---EILQLKNTINKMVDSLQLFAS
PS5H4          TNLTNQVRSIATVTTAVAHGDLSQKIDVHAQG---EILQLKNTINKMVDSLQLFAS
DH5H4          TNLTNQVRSIATVTTAVAHGDLSQKIDVHAQG---EILQLKNTINKMVDSLQLFAS
CA5H4          TNLTNQVRSIATVTTAVAHGDLSQKIDVHAQG---EILQLKNTINKMVDSLQLFAS
YL5H4          SNLTTQVRSIAHVTTAVAQGDLSQKIDVHAQG---EMLALKSTINKMVDRLQVFAS
PS5H2          LNLTNQVREIADVTRAVARGDLSRKINVHAQG---EILQLQMTINTMVDQLRTFAF
DH5H2          LNLTNQVREIADVTRAVARGDLSRKINVHAQG---EILQLQMTINSMVDQLRTFAF
LE5H2          SNLTNQVREIADVTRAVARGDLSRKINVHAQG---EILQLQMTINTMVDQLRTFAF
CA5H2          LNLTNQVREIADVTRAVAKGDLSRKINVHAQG---EILQLQRTINTMVDQLRTFAF
YL5H2          SNLTNQVREIADVTRAVAQGDLSRKINVHAQG---EILELQKTINTMVEQLRTFAF
GM6H2          KELTDNVREIASVTTAVAHGDLTKKIERPARG---EILQLQQTINTMVDQLRTFAS
NC6H2          QNLTDQVREIASVTTAVAHGDLTKKIERPAKG---EILQLQQTINTMVDQLRTFAS
BF6H2          QNLTDQVREIASVTTAVAHGDLTQKIERPAQG---EILQLQQTINTMVDQLRTFAA
MG6H2          QNLTDQVREIASVTTAVAHGDLTQKIESAAKG---EILQLQQTINTMVDQLRTFAS
AN6H2          KNLTDQVREIAAVTTAVAHGDLSQKIESRAQG---EILELQQTINTMVDQLRTFAT
DH5H3          LNLTTQVRNIANVTTAVAKGDLSKKVTADCKG---EILDLKLTINQMVDRLQNFAL
CA5H3          LNLTTQVRNIANVTTAVAKGDLSKKVTADCKG---EILDLKLTINQMVDRLQNFAL
PS5H3          LNLTTQVRNIANVTTAVAKGDLSKKVTADCKG---EILNLKLTINQMVDRLQNFAL
LE5H3          LNLTTQVRNIANVTTAVAKGDLSKKVTADCKG---EILNLKLTINQMVDRLLKFAS
YL5H3          SNLTDQVRNIANVTTAVARGDLSQKVSADCKG---EILDLKSTINKMVDRLQNFAL
GM6H3          NNLTTQVRDIIKVTTAVAKGDLTQKVQADCRG---EIFELKSTINSMVDQLQQFAR
NC6H3          NNLTTQVRDIIKVTTAVAKGDLTQKVQAECRG---EIFELKKTINSMVDQLQQFAR
MG6H3          NNLTTQVRDIINVTTAVAKGDLTQKVQAECRG---EIFELKNTINSMVDQLQQFAR
BF6H3          NNLTTQVRDIAIVTTAVAKGDLTQKVQAECKG---EIKQLKETINSMVDQLQQFAR
AN6H3          NNLTTQVRDIATVTKAVAKGDLTQKVQANCKG---EIAELKNIINSMVDQLRQFAQ
GM6H4          MNLTTQVREIAKVTTAVAKGDLTKKIGVEVKG---EIAELKNTINQMVDRLGTFAV
NC6H4          MNLTTQVREIAKVTTAVAKGDLTKKIGVEVQG---EILDLKNTINTMVDRLGTFAF
BF6H4          MNLTTQVREIAKVTTAVARGDLTKKIEVEVQG---EIASLKDTINTMVDRLSTFAF
MG6H4          MNLTTQVREIANVTSAVAAGDLSKKIRVEVKG---EILDLKNTINTMVDRLGTFAF
AN6H4          NNLTTQVREIADVTTAVAKGDLTKKVTANVQG---EILDLKSTINGMVDRLNTFAF
NC6H5          SNLTSQVRGISTVTQAIANGDMSRKIEVEAKG---EILILKETINNMVDRLSIFCN
MG6H5          SNLTSQVRGISTVTQAIANGDMSRKIDVEAKG---EILILKETINNMVDRLSIFCN
BF6H5          RNLTTQVRGISTVTQAIANGDMSQKIEVAAAG---EILILKETINNMVDRLSIFSN
GM6H5          SNLTVQVRSISTVTQAIANGDMSQKIKVEANG---EIQVLKETINNMVDRLSSFCY
AN6H5          QNLTSQVRSISDVTQAIAKGDLSKKIEVHAQG---EILTLKVTINHMVDRLAKFAT
PS5H5          SNLTSQVRAFAQITAAATDGDFTRFITVEASG---EMDALKTKINQMVLNLRESIQ
DH5H5          SNLTSQVRAFAQITAAATDGDFTRFITVEASG---EMDALKTKINQMVLNLRESIQ
LE5H5          SNLTSQVRAFAQITAAATDGDFTRFITVEALG---EMDALKTKINQMVLNLRESIQ
CA5H5          SNLTSQVRAFAQITAAATDGDFTRFITVEASG---EMDALKTKINQMVFNLRESLQ
YL5H5          ANLTTQVRALVQITAAATDGDFTRFITVEASG---EMDALKTKINQMVLNLRESIQ
NC6H6          NNLTAQVRAFGDITNAATDGDFTKLVEVEASG---EMDELKKKINQMVYNLRDSIQ
MG6H6          NNLTAQVRAFGDITNAATDGDFTKLVEVEASG---EMDELKRKINQMVYNLRDSIQ
BF6H6          NNLTTQVRAFGDITNAATDGDFTKLITVEASG---EMDELKRKINQMVYNLRDSIQ
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GM6H6           SNLTTQVRAFSDITNLATDGDFTKLVDVEASG---EMDELKRKINQMISNLRDSIQ
AN6H6           ENLTSQVRAFGEITDAATDGDFTKLITVNASG---EMDELKRKINKMVSNLRDSIQ
Af1503          STITRPIIELSNTADKIAEGNLEAEVPHQNRA--DEIGILAKSIERLRRSLKVAME
                  :   :  :       : *::   :         *:  .   :: :   *
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**Figure S1. Comparison of individual HAMP domains from NIK1 orthologs from different yeast and filamentous fungi with Af1503 (HAMP domain sequence from *Archaeoglobus fulgidus* protein). Asterisks indicate perfectly conserved residues, two dots indicate very similar residues and full stops indicate similar residues.**