SUPPLEMENTAL MATERIAL

Supplemental Materials and Methods

Overproduction of *NRK2* in the background of GFP-tagged kinesin-2, Kin1p.

We prepared a plasmid containing the *NRK2* coding region fused to the 6xHis epitope tag sequence, and inserted downstream of the MTT1 promoter and between the flanking sequences of the *BTU1* gene (pMTT1-6xHIS-NRK2). To overproduce the 6xHis-NRK2 gene in the background of tagged kinesin-2, GFP-Kin1p expressed at native level, by standard crosses between the strain CU522 and double knockout heterokaryon for two partially redundant kinesin-2 genes, *KIN1* and *KIN2* (Brown *et al*., 1999), we constructed triple mutant heterokaryons which contain the following genotype: *btu1-K350M/btu1-K350M; kin1::neo2/kin1::neo2; kin2::bsr1/kin2::bsr1* (*btu1-K350M/btu1-K350M/btu1-K350M*; *KIN1*, pm-s). Two such heterokaryons were crossed and progenies homozygous for the btu1-K30M, kin1::neo2, kin2::bsr1 alleles in the macronucleus were obtained. Previously we showed that deletion of both *KIN1* and *KIN2* led to inability to grow cilia but the resulting paralyzed cells could be grown on the enriched medium, MEPP (Brown *et al*., 1999). We determined that the presence of the *btu1-k350M* allele in addition to the knockouts of *KIN1* and *KIN2* genes caused synthetic lethality. However, we rescued the dying progeny by biolistic transformation with a fragment encoding GFP-Kin1p (Brown *et al*., 1999) by targeting to the homologous *KIN1* locus. The resulting cells (UG10) carry the btu1-K350M mutation in β-tubulin which confers paclitaxel sensitivity, and are motile due to the expression of GFP-Kin1p. This strain was used to introduce a BTU1-MTT1-6xHis-NRK2-BTU1 fragment into the *BTU1* locus by biolistic transformation and selection of survivors with 20 μM paclitaxel. In the final strain (UG11), the overexpression of 6xHis-Nrk2p can be induced by cadmium in the presence of GFP-Kin1p at its native levels.

Tagging of Nrk1p and Nrk2p in native loci

To produce fragments for tagging the *NRK1* and *NRK2* coding regions in their native loci, pMTT1-NRK1-GFP and pMTT1-NRK2-GFP plasmids were digested with BclI and EcoRV and the resulting NRK1-GFP-3'BTU1 or NRK2-GFP-3'BTU1 fragments were ligated into the pMNBL plasmid carrying the *neo3* cassette (Shang *et al*., 2002), between the SmaI and BamHI sites. These plasmids were digested with XhoI and ApaI and 1.5 kb amplified fragments of the 3' UTR of either *NRK1* or *NRK2* were cloned into these sites to give pNRK1-GFP-3'BTU1-neo3 and pNRK2-GFP-3'BTU1-neo3 plasmids. The above plasmids, had the *neo3* selectable marker cassette embedded into the 3' UTR of either GFP-tagged *NRK1* or *NRK2* coding region. The inserts of pNRK1-GFP-BTU1-neo3 and pNRK2-GFP-BTU1-neo3 were separated by digestion with ApaI and SacI restriction endonucleases. The constructs were targeted to the native loci by biolistic transformation of starved wildtype CU428 *Tetrahymena* cells (Cassidy-Hanley *et al*., 1997). Cells subjected to bombardment were incubated overnight in 10 mM Tris-HCl pH 7.5, and transferred to SPP medium with $CdCl_2$ (1μg/ml) and after 2 hrs selection with paromomycin (120 μg/ml) was initiated.

NRK1 and _NRK2_ single and double germline gene knockouts

We cloned the 4.7 kb macronuclear DNA fragment of *NRK1* flanked by SpeI and PstI sites. For the *NRK2* gene, we cloned its 4.8 kb HindIII genomic fragment. For the *NRK1* knockout, the p4T21 plasmid was digested with EcoRV and BamHI to isolate the *neo2* cassette (Gaertig *et al.*, 1994). A plasmid with the 4.7 kb genomic fragment containing *NRK1* was digested with BamHI, blunt ends were made with T4 DNA polymerase, digested with BglII and ligated to the *neo2* fragment. As a result, 122 bp of the 5' UTR region and 1 kb of the coding region of *NRK1* were replaced by *neo2*, inserted in a reverse transcriptional orientation. To prepare a knockout plasmid construct for *NRK2*, the p4B21 plasmid (Brown *et al.*, 1999) was digested with SmaI and EcoRV to obtain a blunt-ended *bsr1* cassette. A plasmid with the 4.8 kb genomic fragment of *NRK2* was digested with NdeI to remove 1.37 kb of the *NRK2* coding region. Blunt ends were created with T4 DNA polymerase and the resulting fragment used for insertion of the blunt-ended *bsr1* cassette in a reverse transcriptional orientation. For germline transformation, the *Tetrahymena* cells of the CU428 and B2086 strains (provided by Dr. Peter Bruns, Cornell University) were induced to mate and subjected to biolistic transformation between 3-4 hrs after strain mixing as described (Cassidy-Hanley *et al.*, 1997). Bombarded cells were incubated in 50 ml of SPPA for 4 –5 hrs followed by paromomycin (120 μg/ml) or blasticidin S selection (60 μg/ml). Single and double knockout heterokaryons and homokaryons were created as described (Hai *et al.*, 1999).

Immunofluorescence

The imunofluorescence studies were done as described in the Materials and Methods of the main section except that rabbit polyclonal anti-GFP antibodies (ab6556, from Abcam) were used at the 1:600 dilution.

Supplemental Figure Legends

Figure S1. Expression of Nrk1p-GFP, Nrk2p-GFP and effect of expression of NRKs on cell proliferation. A-B. Western blots showing levels of either Nrk1p-GFP (A) or Nrk2p-GFP (B) as a function of time after addition of cadmium chloride to induce the transgenes. C. Growth curves of cell populations carrying several NRK transgenes after cadmium chloride induction.

Figure S2. Left panel, immunofluorescence image with an anti-centrin 20H5 antibody of a wildtype cell prior to cytokinesis. Note the properly assembled membranelles of the new oral apparatus located below the cleavage furrow region. Middle panel, detection of NRK2p-GFP in overproducing cell prior to cytokinesis displaying a highly disorganized oral apparatus to which Nrk2p-GFP localizes . Right panel, an Nrk17p-overproducing cell arrested in cytokinesis with a cleavage furrow shifted to the anterior end (signal of Nrk17p-GFP).

Figure S3. Immunofluorescence images documenting the localizations of either Nrk1p-GFP (B-C) or Nrk2p-GFP (D-E) expressed in their native loci. The polyclonal anti-GFP antibody (Abcam) was used and gives a slight nonspecific labeling of ciliary tips and basal bodies in cells lacking an expressed GFP (A). Nrk1p-GFP was detected in newly formed short locomotory cilia (arrows in B) and in assembling oral cilia in dividing cells (arrows in C). Nrk2p-GFP was not detected in non-dividing cells. In dividing cells, Nrk2p was detected in assembling oral cilia (arrow in D). At a more advanced stage of cell division, Nrk2p-GFP was detected in a subset or anterior and ventral cilia (arrow in D). In all cilia, there Nrk2p was more concentrated at the tips of cilia.

Figure S4. A cell which expresses a GFP tagged kinesin-2, GFP-Kin1p in the native locus and overexpresses 6xHis-Nrk2p under the MTT1 promoter, 2.5 hrs after induction with 3 μg/ml $CdCl_2$, labeled by immunofluorescence using anti-GFP antibodies (Abcam) (left panel) and anti-polyglutamylated tubulin antibody, ID5, that is used here as a marker of cilia (middle panel). The merged image of GFP and polyglutamylated tubulins is shown in the right panel. The magnified portions of the anterior and posterior area of the cell are shown above and below. The large arrow indicates the anterior region enriched in short cilia which have increased level of GFP-kin1p. The small arrow shows posterior cilia which do not undergo shortening and also have a significantly lower signal of kinesin-2.

Figure S5. RT-PCR shows that most if not all NRK genes of *Tetrahymena thermophila* are expressed. We performed RT-PCR to detect gene products for a total of 16 predicted NRK genes including 8 paralogs within the CNK2 subtype. For each NRK gene we performed PCR reactions using total cDNA made from RNA of vegetative cells (left lane), RNA from conjugating cells (middle lane) and genomic DNA (right lane).

Whenever possible we used gene-specific primers flanking an intron which resulted in amplification of a larger fragment using genomic DNA. Bands were produced using cDNA for all 13 sets of primers indicating that all paralogs of CNK2 type are expressed. Furthermore, the absence of an intron-containing fragment in the cDNA-based reactions indicates lack of contamination of cDNA by genomic DNA.

Figure S6. A multiple alignment of NRK kinase domain sequences. Note that for the tree construction (Fig. 1A) we did not include lineage-specific insertions. *Homo sapiens*: Nek1 (NP_036356.1), Nek2 (NP_002488.1), Nek3 (NP_689933.1), Nek4 (NP_003148.1), Nek6 (AAG13417.1), Nek7 (NP_598001.1), Nek8 (NP_835464.1), Nek9 (AAL87410.1), Nek11 (NP_079076.2), aurora kinase (NP_004208.1), cdk2 kinase (CAA43985.1), PKC1beta kinase (CAA29634.1). *Mus musculus*: CAMK kinase (NP_598687.1). *Drosophila melanogaster*: CG17256-PA (NP_572415.1), CG10951-PA, (NP_651293.1). *Caenorhabditis elegans*: 1C941 (NP_490967.1), ZC581.1 (AAB54139.1), F19H6.1 (CAA92169.2). *Chlamydomonas reinhardtii*: FA2 (AAL86904.1), Cnk1p (AAQ64682.1), Cnk2p (AAQ64683.1), Cnk3p (AAQ64684.1), Cnk4p (AAQ64685.1), Cnk5p (AAQ64686.1), Cnk6p (AAQ64687.1). *Saccharomyces pombe*: Fin1 (O13839). *Saccharomyces cerevisiae*: KIN3 (NP_009410.1). *Aspergillus nidulans*: NIMA (EAA60031.1). *Neurospora crassa*: NIM1 (XP_330623.1). *Crithidia fasciculate*: CfNRK (CAD38824.1). Loci ID numbers for the *T. thermophila* are: *NRK1* (10831), *NRK2* (2469), *NRK3* (18448), *NRK4* (24991), *NRK5* (19380), *NRK6* (7740), *NRK7* (19674), *NRK8* (1405), *NRK9* (17999), *NRK10* (2122), *NRK11* (28433), *NRK12* (28502), *NRK13* (3425), *NRK14* (29340), *NRK15* (29341), *NRK16* (29473), *NRK17*

(22242), *NRK18* (18676), *NRK19* (4647), *NRK20* (20809), *NRK21* (15435), *NRK22* (24902), *NRK23* (26810), *NRK24* (13547), *NRK25* (29341), *NRK26* (15734), *NRK27* (22348), *NRK28* (8589), *NRK29* (1112), *NRK30* (20296), *NRK31* (23433), *NRK32* (21625), *NRK33* (21626), *NRK34* (24670), *NRK35* (1924), *NRK36* (6348), *NRK37* (2218), *NRK38* (17542), *NRK39* (3979).

Figure S7. A partial multiple alignment of known and predicted CNK2 subfamily NRKs from various protists (Vc: *Volvox carteri*; T.pyr: *Tetrahymena pyriformis*; FN0…:*Paramecium tetraurelia*). Note the presence of conserved amino acid motifs located downstream of the kinase domain region.

References for supplemental materials

Brown, J.M., Marsala, C., Kosoy, R., and Gaertig, J. (1999). Kinesin-II is preferentially targeted to assembling cilia and is required for ciliogenesis and normal cytokinesis in *Tetrahymena*. Mol. Biol. Cell *10*, 3081-3096.

Cassidy-Hanley, D., Bowen, J., Lee, J., Cole, E.S., VerPlank, L.A., Gaertig, J., Gorovsky, M.A., and Bruns, P.J. (1997). Germline and Somatic Transformation of Mating *Tetrahymena thermophila* by Particle Bombardment. Genetics *146*, 135-147.

Gaertig, J., Gu, L., Hai, B., and Gorovsky, M.A. (1994). High frequency vector-mediated transformation and gene replacement in *Tetrahymena*. Nucleic Acids Res. *22*, 5391-5398.

Hai, B., Gaertig, J., and Gorovsky, M.A. (1999). Knockout heterokaryons enable facile mutagenic analysis of essential genes in *Tetrahymena*. Methods Cell Biol. *62*, 513-531.

Shang, Y., Song, X., Bowen, J., Corstanje, R., Gao, Y., Gaertig, J., and Gorovsky, M.A. (2002). A robust inducible-repressible promoter greatly facilitates gene knockouts, conditional expression, and overexpression of homologous and heterologous genes in Tetrahymena thermophila. Proc Natl Acad Sci *99*, 3734-3739.

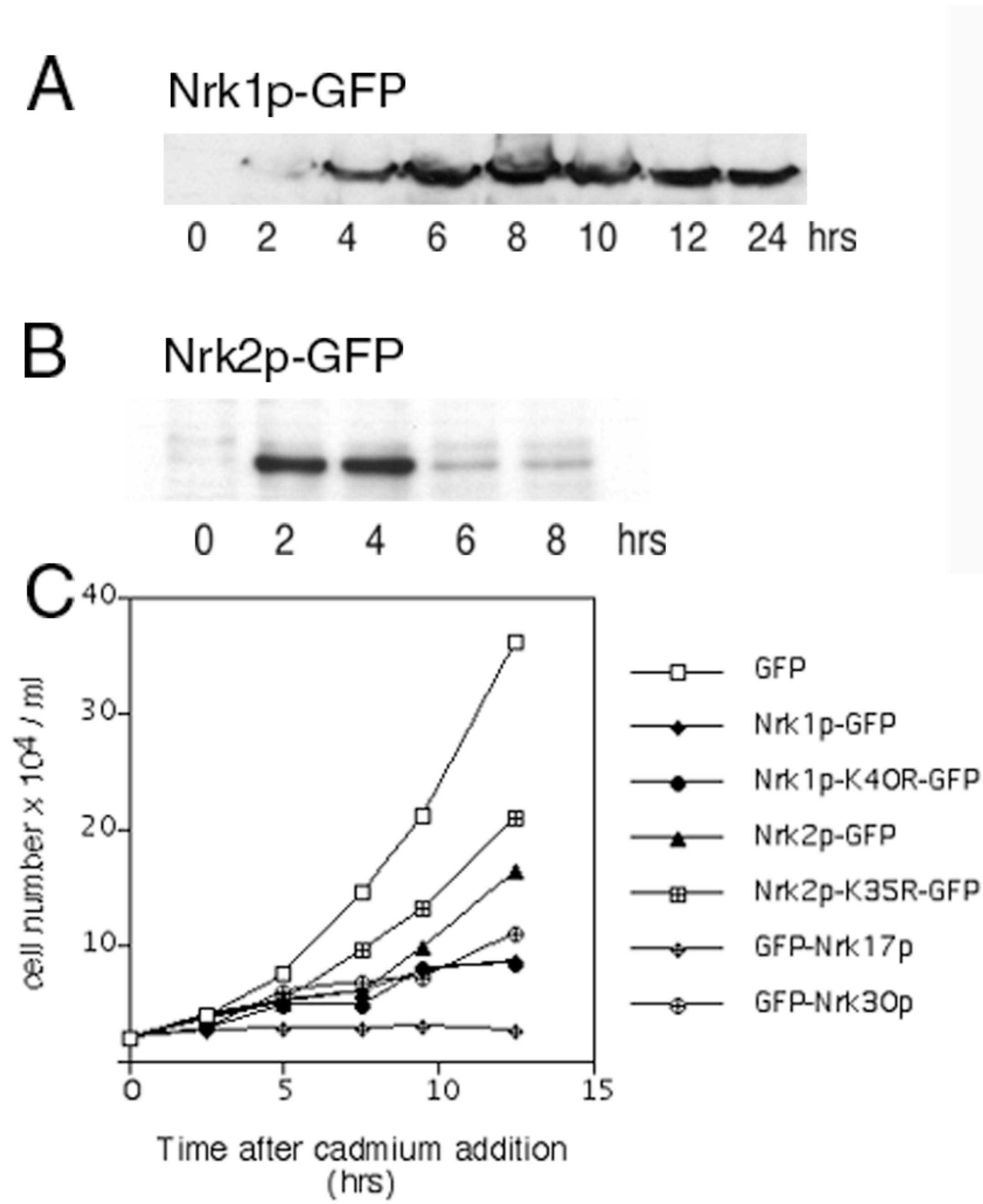

A
Nrk1p-GFP
0 2 4 6 8 10 12 24 hrs
B
Nrk2p-GFP
0 2 4 6 8 hrs
C
cell number x 10⁴ / ml
40
30
20
10
0
5
10
15
Time after cadmium addition (hrs)
GFP
Nrk1p-GFP
Nrk1p-K40R-GFP
Nrk2p-GFP
Nrk2p-K35R-GFP
GFP-Nrk17p
GFP-Nrk30p

Figure S1.

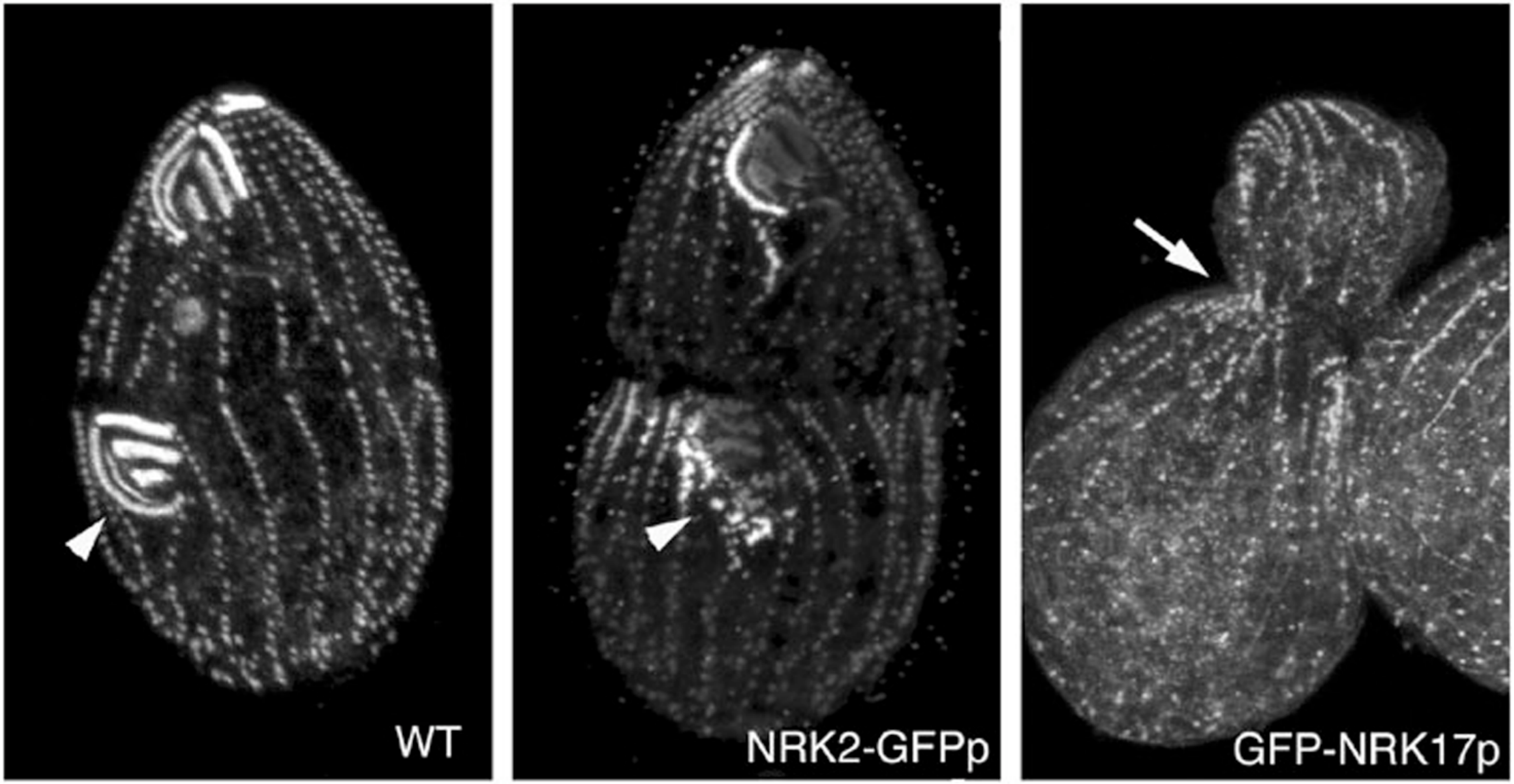


Figure S2

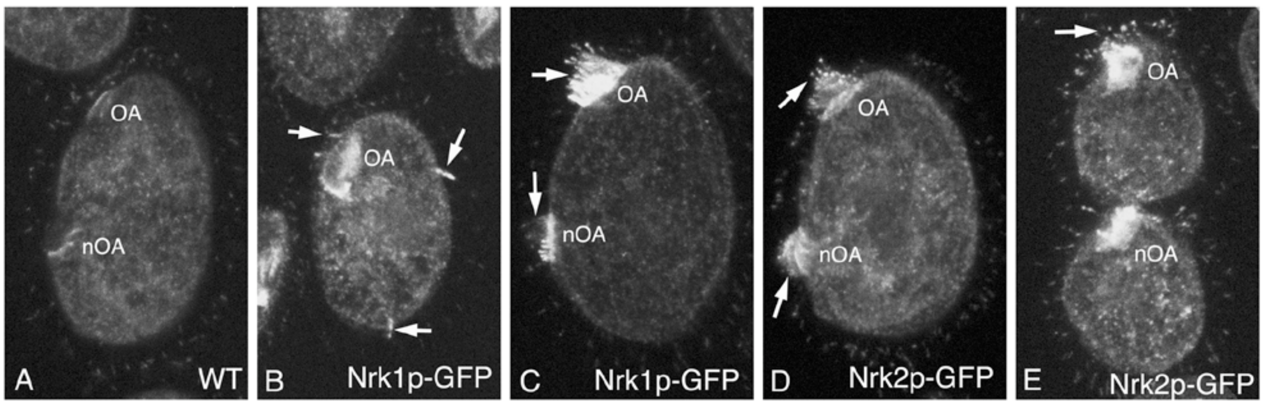

OA
nOA
A
WT
OA
B
Nrk1p-GFP
OA
nOA
C
Nrk1p-GFP
OA
nOA
D
Nrk2p-GFP
OA
nOA
E
Nrk2p-GFP

Figure S3

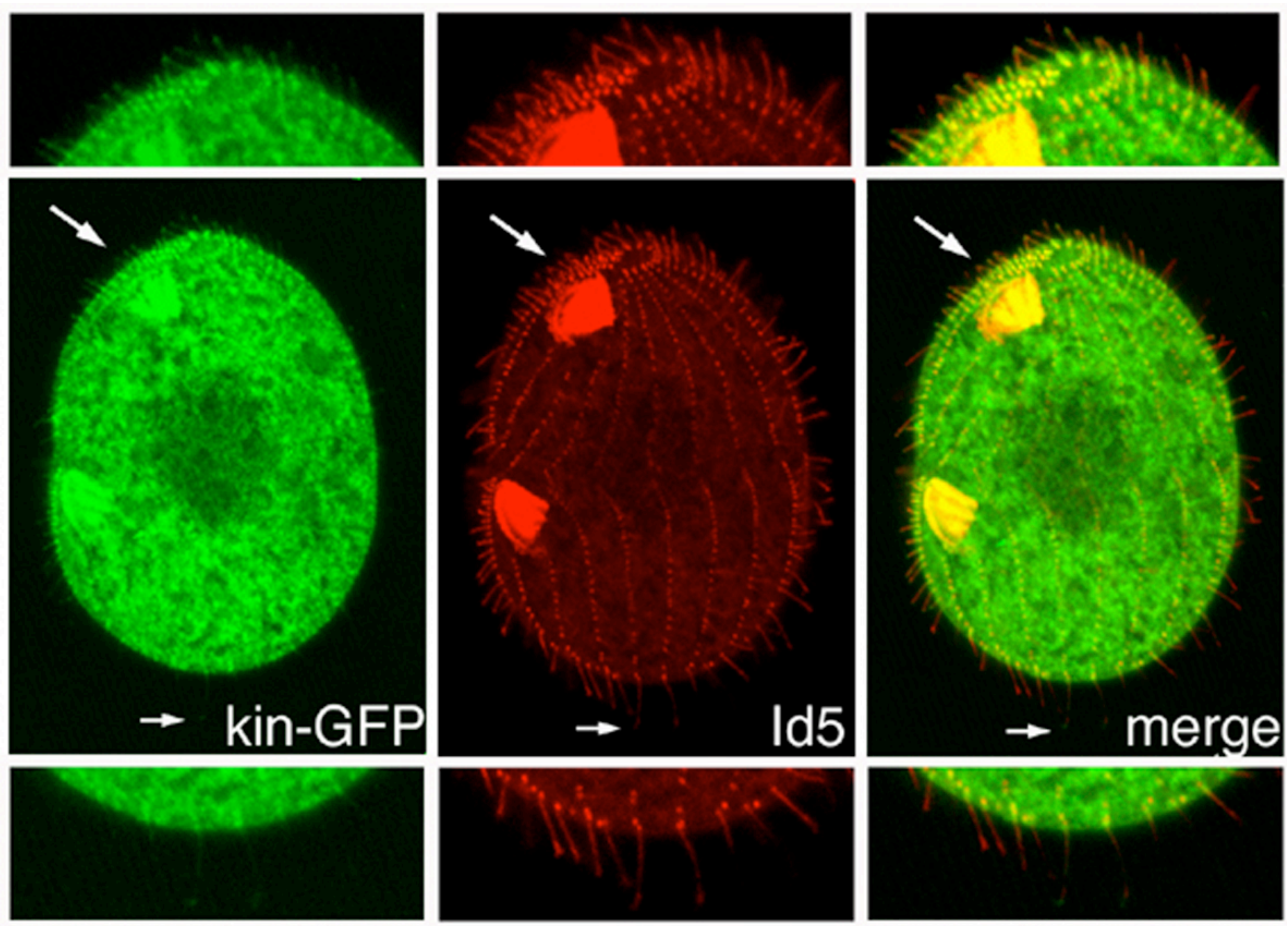
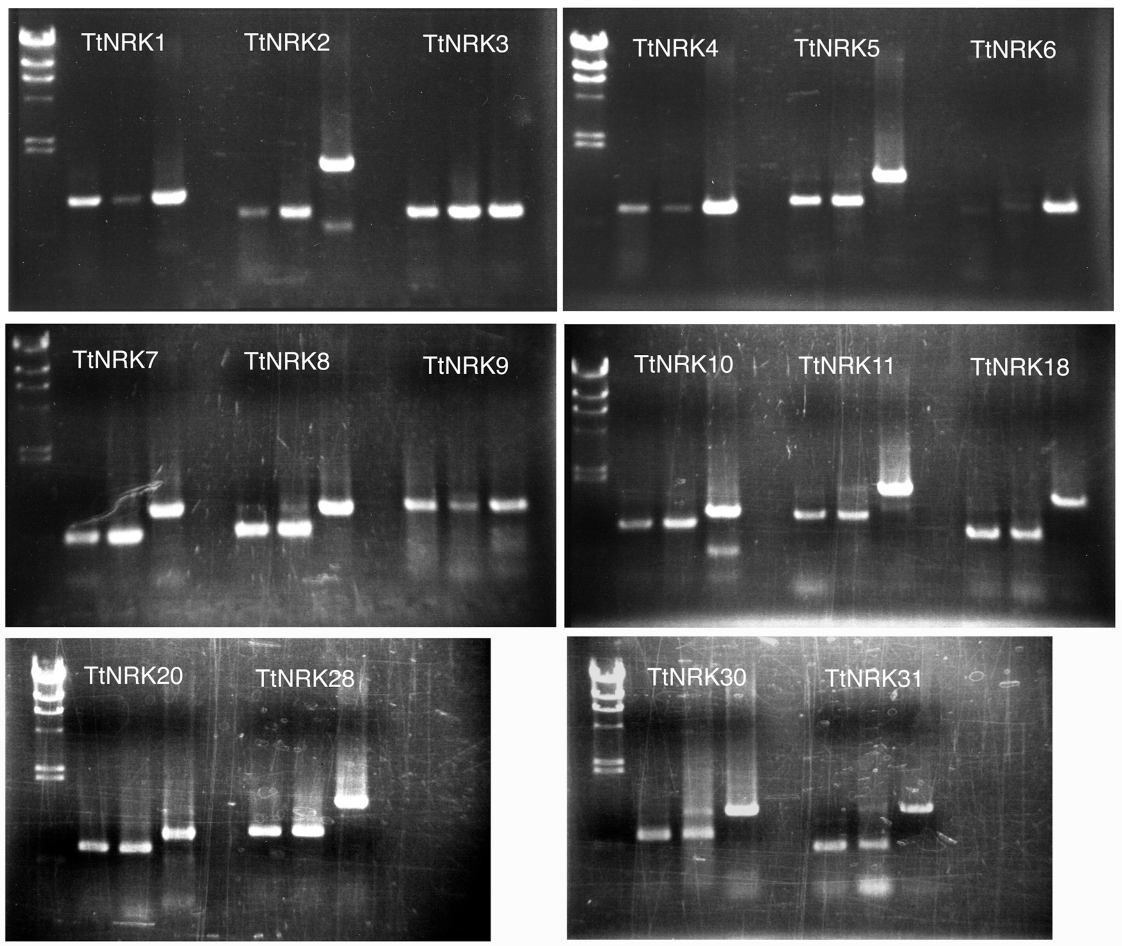


Fig. S4



TtNRK1
TtNRK2
TtNRK3
TtNRK4
TtNRK5
TtNRK6
TtNRK7
TtNRK8
TtNRK9
TtNRK10
TtNRK11
TtNRK18
TtNRK20
TtNRK28
TtNRK30
TtNRK31

Fig. S5

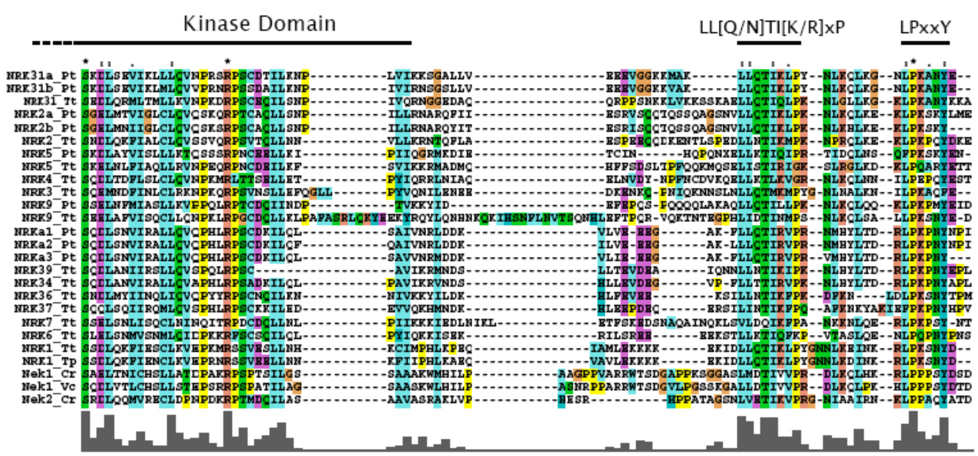


Fig. S7

Fig. S6

CLUSTAL W (1.7) multiple sequence alignment

```
NEK8-H.sapiens                YERIRVVGRGAFGIVHLCLRKADQK----LVIIKQIPVEQM-----TKEERQAAQNECQV
Nek8-2-C.elegans              YEKVRVVGRGAFGVCWLCRGKNDASHQ--KVIIKLINTHGM-----TEKEENSIQSEVNL
Nek8-1-C.elegans              YERIRTVGKGAFGSAVLYRRREDSS----LVIIKEINMYDL-----DSSQRRLALNEVSL
Nek8-similar-D.melanogaster   YEKVRVVGQGSFGIAILYRRKSDGH----QIVFKQINLSEL-----SPPGRDLAMNEVDV
NEK9-_Nercc1                  YIPIRVLGRGAFGEATLYRRTEDDS----LVVWKEVDLTRL-----SEKERRDALNEIVI
NRK18-T.thermophila           YEILGQIGKGSFGLVQKIKRKSDGK----VLVWKEMNYGRM-----SEREKQQLVAEVNI
NRK20-T.thermophila           YEVIGEIGAGQFGKVVKIKRKSDNK----ILVWKQLEYGKM-----DEKEKSQLVAEVNI
NEK2-H.sapiens                YEVLYTIGTGSYGRCQKIRRKSDGK----ILVWKELDYGSM-----TEAEKQMLVSEVNL
Nek2-similar-D.melanogaster   YEVLAVMGNGSFGTCYKVRDKSTGE----LFAWKGMNYDEL-----DEAKCDALVSEISV
NIM-1-N.crassa]               YELLEKIGHGSFGIIRKVRRKADGM----ILCRKEISYLKM-----SQKEREQLHAEFSI
NIMA-A.nidulans               YEVLEKIGCGSFGIIRKVKRKSDGF----ILCRKEINYIKM-----STKEREQLTAEFNI
KIN3-S.cerevisiae_            YQVLEEIGRGSFGSVRKVIHIPTKK----LLVRKDIKYGHM-----NSKERQQLIAECSI
Fin1-S.pombe                  YKILECIGHGSFGRIYKVQRLKDGA----LLAQKEIHFGNI-----TRQEKQYIADEVNI
NEK6-H.sapiens                FQIEKKIGRGQFSEVYKATCLLDRK----TVALKKVQIFEMM----DAKARQDCVKEIGL
NEK7-H.sapiens                FRIEKKIGRGQFSEVYRAACLLDGV----PVALKKVQIFDLM----DAKARADCIKEIDL
Nek7-similar-C.elegans        FIIEKKIGKGQFSEVFRAQCTWVDL----HVALKKIQVFEMV----DQKARQDCLKEIDL
Nrk6-Chlamydomonas            YDVQKPVGKGGYAVVYKGIRRDDGR----VVAVKKVEIFEM-----SAKKRDRCLQEVTL
NRK1-T.thermophila            FEILKRLGEGSFGSVYQVKRKSDEK----IYAMKKVKMMSL-----STKEKENALNEVRI
NRK3-T.thermophila            FNVQKTLGNGAFSWVYKVQRKQDGQ----VYALKKVKLREL-----SYKEKENALNEIRI
NRK2-T.thermophila            FVNLQKIGEGSYSSVHKVRRISDNQ----EYALKKVKLSGL-----SEKEKDNALNEIRI
NRK31-T.termophila            FEVISKLGEGSFSQVFQVKRKSDGM----IYAMKKVKMGLL-----KEKEKENALNEVRI
NRK4-T.thermophila            FDVIRKLGEGAYSSVFKVRKISNGQ----DYAMKNIKMGSL-----SAKEQENAINEVRF
NRK5-T.thermophila            FEILSKLGEGSFSTVYRVRRGKDGK----EYALKRIKMMKL-----NEKERENAVNEVRF
NRK7-T.thermophila            FDIIKTLGEGSFAKVYKVVRKSDGQ----SYAMKRCKIGLM-----KQRDKDNALNEVRI
NRK9-T.thermophila            FKILCKLGEGSFSTVFKVLRLVDNK----IYAMKKVQMSRL-----NEKEKGNSLNEIRI
NRK6-T.thermophila            FEIIEKLGEGAYSQVYKVKRTIDQQ----IYALKKVNLTNL-----SLKEKENALNEVRI
Cnk1-Chlamydomonas            FKVHKLLGKGSYGKVYKVERESDKQ----LYALKEADLGSM-----SQAERADAVNEVRL
Cnk2-Chlamydomonas            FKVLKFLGKGSYGSVFLVQRLADSQ----TYALKEMDVRSM-----SQAEREDSINEIRL
NRK11-T.thermophila           YDNLVKLGQGSYGVVYKGRKKSDGK----TYVIKEINMKFM-----DQKQKQDAVNEGNL
NRK17-T.thermophila           FDTLGKLGQGSFGVVYKVKRVVDGN----VYVMKQINISKM-----NSRMKQDAINEVHI
NRK8-T.thermophila            FEILKKLGQGAHGVVYKVRRKKDQN----TYVLKQILAGGM-----QQKQRKECINEAIL
FA2-Chalmydomonas             YELQY-IDKGSFGAVFKAVRKSDGR----VYALKQVDLRSADFK--NPTLDRAAAIDEAR
Nrk4-Chlamydomonas            FIIKEKIGSGSYGVVFKVVRKVDKH----VYAMKEIDLQGM-----SRKEQEECIRETRV
NEK1-H.sapiens                YVRLQKIGEGSFGKAILVKSTEDGR----QYVIKEINISRM-----SSKEREESRREVAV
NEK3-H.sapiens                YMVLRMIGEGSFGRALLVQHESSNQ----MFAMKEIRLPK------SFSNTQNSRKEAVL
NRK26-T.thermophila           YTKIKVVGKGSFGYAVLVQSNTENNKK--YYVIKIIDISKM-----DRKQREEALNEVHV
NRK29-T.thermophila           YIKIELVGKGNFGLAVLVQSKINRK----YYIMKVYIFQQTIIE--FLKFQQDALNEVKF
NEK4-H.sapiens                YCYLRVVGKGSYGEVTLVKHRRDGK----QYVIKKLNLRNA-----SSRERRAAEQEAQL
NRK22-T.termophila            YETVKLIGSGAFGQVYLVKHKREDK----MYVNKKIKTRDM-----SQKDRENTENEVRL
NRK10-T.thermophila           YRELEQIGKGTSGTVFLVKSKQDKK----FYIAKKIILTNL-----NEQERQAVEQELIL
NRK30-T.thermophila           YKEIELIGRGTQGSAMLVECRSDKK----RYVSKKVILTNL-----SEKDQNNAIQELKL
NRK13-T.thermophila           YKEIEVIGRGNFGSATLVEKLDNKK----QYIAKKIVLSSL-----NPKQQDSALQEAQL
Cnk5-Chlamydomonas            YLDLTAIGQGQYGTAYRAKDKYDNQ----LYCIKRIPMS-------AKDDHAGALREAQL
NEK11-H.sapiens               YVLQQKLGSGSFGTVYLVSDKKAKR----GEELKVLKEISVGEL--NPNETVQANLEAQL
NRK19-T.thermophila           YKRLKLLGEGSFGKAYLVESQSDKS----KWVIKQISLDAM-----SPEEKKESYKEAKI
NRK21-T.thermophila           YKRIKLLGEGAFGKAYLVEDLRTHE----LLVQKQMDMKAM-----STEEKRETQKEARI
NRK12-T.thermophila           YKQIKLLGEGSFGKAYLVECQSDGS----LCVIKKMDTKSM-----TEAEKQETVREAHI
NRK27-T.thermophila           YKKIKLLGQGTYGKAFLVERKSDGL----KCVIKQIEMSHM-----TEEERKEAQKEANI
NRK15-T.thermophila           YLWIKQIGYGSQGQVHLIESKEDSS----KLAIKNISTKNM-----SQQQKRKHQDEYRC
NRK25-T.thermophila           YVLKKKIGQGNYGQVYLVQSQRDGS----ILAIKNISTQNM-----IFEDLEKCLKECTI
NRK14-T.thermophila           YRWIKKIGEGAYGQVHLVQSIRDGS----KWAIKNIYVSG------GEDVKKKRQSEYKI
NRK16-T.thermophila           YKVLKTLGQGASGSVELVQK-NDGN----LYALKTISMKYM-----NDTEKRSAQSEVTL
NRK24-T.thermophila           YKVIRMIGQGAGGSVELVQKRSDGQ----LFALKTISMKFM-----DDQQKKMAQQEITL
Cnk3-Chalmydomonas            YELQREIGSGSYGQAVLATRLEDGM----QVVCKQIRLFEM-----DDKARADTLTEAKV
Nrk-C.fasciculata             YVLTTLVGRNPTTAAFVATRGSDPSE---KVVAKFVMLN-------DDKQATYARSELHC
```

```
CAMKK-M.musculus             YDFRDVLGTGAFSEVILAEDKRTQK----LVAIKCIAKKAL------EGKEGSMENEIAV


NEK8-H.sapiens               LKLLNHPNVIEYYENFLEDK-----ALMIAMEYAPGGTLAEFIQKRC-------------
Nek8-2-C.elegans             LKKVQHPLIIGYIDSFIMDN-----QLGIVMQYAEGGTLERLINDQR-----------AI
Nek8-1-C.elegans             LSRIEHPNIIAYYDSFEEEG-----VLMIEMEYADGGTLAQMLSRTQ-------------
Nek8-similar-D.melanogaster  FSKLHHPNIVSYLGSFIKDN-----TLLIEMEYADGGTLAQIIAERQ-------------
NEK9-_Nercc1                 LALLQHDNIIAYYNHFMDNT-----TLLIELEYCNGGNLYDKILRQK-------------
NRK18-T.thermophila          IRELKHPNIVRYYDRIIEKKDT---KIYIIMEYCEGGDVGTLLKKCK-------------
NRK20-T.thermophila          LREIRHPNVVRYYDRIIDKQNQ---HIYIIMEYCEGGDLAAFLKNLK-------------
NEK2-H.sapiens               LRELKHPNIVRYYDRIIDRTNT---TLYIVMEYCEGGDLASVITKGT-------------
Nek2-similar-D.melanogaster  LRQLQHPNIVQYYHHLVNREAK---SVYIVMECCAGGDLAQIVQRAR-------------
NIM-1-N.crassa]              LSTLRHPNIVGYYHREHLKATQ---DLHLYMEYCGNGDLGRVIRNLI-------------
NIMA-A.nidulans              LSSLRHPNIVAYYHREHLKASQ---DLYLYMEYCGGGDLSMVIKNLK-------------
KIN3-S.cerevisiae_           LSQLKHENIVEFYNWDFDEQKE---VLYLYMEYCSRGDLSQMIKHYK-------------
Fin1-S.pombe                 LRNLKHPNIVQYCGEELNRSAQ---VINLYMEYCGHGDLANLIQRYK-------------
NEK6-H.sapiens               LKQLNHPNIIKYLDSFIEDN-----ELNIVLELADAGDLSQMIKYFK-------------
NEK7-H.sapiens               LKQLNHPNVIKYYASFIEDN-----ELNIVLELADAGDLSRMIKHFK-------------
Nek7-similar-C.elegans       LKQLNHVNVIRYYASFIDNN-----QLNIVLELAEAGDMSRMIKHFK-------------
Cnk6-Chlamydomonas           LQQLDHPNIIQMLDAFIDEN-----MLIIIFEWAPAGDLKRLIKKTA-------------
NRK1-T.thermophila           LASIKSDNIISYKEAFYDEKSS---TLCIIMEFATKGDVLQQISEKK-------------
NRK3-T.thermophila           LASINSPHIIRYKDAFYDNASG---CLCIVMEYAENGDLMAKLQDYK-------------
NRK2-T.thermophila           LASIAHPNMIAYKDAFFDESSH---SLCIVMELAVNGDLSKKIDSAK-------------
NRK-31-T.termophila          LASLNDEFIVGYKEAFIDEQSQ---ILCVVMEYAAGGDLQGKITANI-------------
NRK4-T.thermophila           LASLSSQEIIGYKEAFYDEKTG---LLHIIMEYCAGGDLLNKIRNLK-------------
NRK5-T.thermophila           LASINCRNIISYKQAIYDEGVN---QLCVIMEYAEGGDLARIIRHAS-------------
NRK7-T.thermophila           LASIKNQYVIAYKEAIYDEQSE---CLFVIMEYAAGGDLHQQVKSCI-------------
NRK9-T.thermophila           LASIKCDHIIEYRDSFFDDQSD---TLCIVMEYAGSGDLLQKLKEYKLINEKLLEDGQNE
NRK6-T.thermophila           IASIRHPNVVSFKESFLSDDGE---FLYLVMEYADDGDVLEKIKKHI-------------
Cnk1-Chlamydomonas           LVSITHHNVIRYNEAFLLGN-----KLCTVMEYAPFGDLRYYISKGA-------------
Cnk2-Chlamydomonas           LASVNHPNVVCYNEAFLDGN-----RLCIIMEYAADGDLAKVIKKQQ-------------
NRK11-T.thermophila          LKHLESPYVVKYYDMFIEQN-----DLYIVMEFCENGDLLQYIKKQK-------------
NRK17-T.thermophila          LSKLNNPYVVKYYDSFVDKN-----LLCIVMEYCDSGDLSSFIKSQL-------------
NRK8-T.thermophila           LNKLNSPYIVRYYDSFLENN-----QLCIVMEYCEQGDLENFIKNQM-------------
FA2-Chlamydomonas            MLAQLNHPHVIRHFESFVDGEG---KLNILMEYASKGSVRQLVKSYR-------------
Nrk4-Chlamydomonas           LSSLDSDFIIRYYDSFLEKG-----KLYIITEYAANGNLHDYIKKQK-------------
NEK1-H.sapiens               LANMKHPNIVQYRESFEENG-----SLYIVMDYCEGGDLFKRINAQK-------------
NEK3-H.sapiens               LAKMKHPNIVAFKESFEAEG-----HLYIVMEYCDGGDLMQKIKQQK-------------
NRK26-T.thermophila          LKAMKHPYIITYRESFIEKR-----CLCIIMEYAQGGDLYTKIAKQK-------------
NRK29-T.thermophila          LKELGHPFIIAYRESFVDKDR----YLCIVMDYCEEGDLYNQIIEQK-------------
NEK4-H.sapiens               LSQLKHPNIVTYKESWEGGDG----LLYIVMGFCEGGDLYRKLKEQK-------------
NRK22-T.termophila           LQKLRHANIVAYKDSYMDREQ----YLNIVMIHCEGGDMHNRIKNQK-------------
NRK10-T.thermophila          LKKLKHPHIVGYKENFLEPY-----YMIIIMEYCEQGDLSFHIKQKL-------------
NRK30-T.thermophila          LREMKHPNIVKFIESYKEKN-----KVIIIMEYCEYGDLSQLIKQKS-------------
NRK13-T.thermophila          LKDLNHKNIVSYIESFKEED-----LLIIIMEYCEHGDLAFHIKRKK-------------
Cnk5-Chlamydomonas           LDSLDHPNIIRYRESFVDKDG----SLCIVTSFCEEGDLFNRIRKKA-------------
NEK11-H.sapiens              LSKLDHPAIVKFHASFVEQD-----NFCIITEYCEGRDLDDKIQEYK-------------
NRK19-T.thermophila          LEQLNHPNIVKFKEIYKTKSG----KLCIVMEYADGGDLSQKIQKQR-------------
NRK21-T.thermophila          LQQLNHPNIVKFKDVYTTKKG----KLCIIMEYADGGDLAKVVKDAR-------------
NRK12-T.thermophila          LEALNHPCIVKFREVYKTKK-----ALCIVMDFCDGGDLAKKIQDYK-------------
NRK27-T.thermophila          LQMLNHHNIIKYHEQYKTKKG----RLCIVMDYAEGGDLNQVITKAR-------------
NRK15-T.thermophila          LQKLDH--IVKFKKAIHCINNQ---MIQIIMEYTEGGDLQKAIEKQI-------------
NRK25-T.thermophila          LQKLDNPHIVKFKKAINQPNKQ---MIYIMMEYAEGGDLRKKIKEQK-------------
NRK14-T.thermophila          LKKLDNPHIIKFKEAINYRNQQ---LIQIIMEYTDGGDLEKLIEKQK-------------
NRK16-T.thermophila          LKVLVAPTIIRYYEAFVQND-----SIYIVMEYAKEGALSDKIQEHK-------------
NRK24-T.thermophila          LKVLVAPSIIRYYESFVEND-----SIHIIMEYAKEGALSDKITEHK-------------
Cnk3-Chalmydomonas           LAQFNHVNIVHYYECVLESG-----VLNIVMEYANGGDLAAAIQRRQ-------------
Nrk-C.fasciculata            LAACTHFGIVKHYDDFKSED-----KLLLIMEYGSGGDLNKQIKQRL-------------
```

```
CAMKK-M.musculus              LHKIKHPNIVALDDIYESGG-----HLYLIMQLVSGGELFDRIVEKG-------------


NEK8-H.sapiens                -----NSLLEEETILHFFVQILLALHHVHTH----------------------------L
Nek8-2-C.elegans              KDSNMREYFPEKTVLDYFTQILIALNHMHQK----------------------------N
Nek8-1-C.elegans              ------NLLDEEQIGDMMIQMCSAVAYLHEN----------------------------S
Nek8-similar-D.melanogaster   ----GKLHFPERYIIAVFEQISSAINYMHSE----------------------------N
NEK9-_Nercc1                  -----DKLFEEEMVVWYLFQIVSAVSCIHKA----------------------------G
NRK18-T.thermophila           ---KEKDYIAEDVIWKIFTQIILALNECHNR-------------------------PQGK
NRK20-T.thermophila           ---KEKETIPEEAIWRIFMQIVLALHEIHHK----------------------------K
NEK2-H.sapiens                ---KERQYLDEEFVLRVMTQLTLALKECHRR-----------------------SDGGHT
Nek2-similar-D.melanogaster   ---SQRQRFEEPYIWRVLFQLCRALQVCHNK------------------------IPNGT
NIM-1-N.crassa]               ---KNNQYAEESFVWSIFSQLVTALYRCHYG--VDPPEVGKTVLGLGSTARPKPPSGGMT
NIMA-A.nidulans               ---RTNKYAEEDFVWRILSQLVTALYRCHYG-TDPAEVGSNLLGPAPKPSGLKGKQAQMT
KIN3-S.cerevisiae_            ---QEHKYIPEKIVWGILAQLLTALYKCHYG-------VELPTLTTIYDRMKPPVKGKNI
Fin1-S.pombe                  ---EEKKRFTEQEVLKFFTQLLLALYRCHYG---------ENAPACDSQWPREIFHPKQS
NEK6-H.sapiens                ---KQKRLIPERTVWKYFVQLCSAVEHMHSR----------------------------R
NEK7-H.sapiens                ---KQKRLIPERTVWKYFVQLCSALEHMHSR----------------------------R
Nek7-similar-C.elegans        ---KGGRLIPEKTIWKYFVQLARALAHMHSK----------------------------R
Cnk6-Chlamydomonas            ---EQGKTLDEPSIWTLFYQVTDGLRYMHQH----------------------------R
NRK1-T.thermophila            ---KKHSYFEENLIWKYAADMLLGLKSLHDM----------------------------K
NRK3-T.thermophila            ---KRNMFMDEAKIWKYAAQILLGLKSLHDL----------------------------K
NRK2-T.thermophila            ---KRNSFVPEEEIWTVALHMLRGLKAMHSK----------------------------K
NRK-31-T.termophila           ---KSKTMFPESEVWKALIHMSKGLQILHQM----------------------------Q
NRK4-T.thermophila            ---KKNQYLDEKVVWLYIIQMIKGLKCLHDL----------------------------N
NRK5-T.thermophila            ---KAGKYIEEDMIWSYAIQMTIGIKALHDL----------------------------N
NRK7-T.thermophila            ---KSKTYLDENQIWIWTIQMLYGLKALHDL----------------------------K
NRK9-T.thermophila            TSESQMVFMDEELIWDYFIQCLKGLKCLHDL----------------------------K
NRK6-T.thermophila            ---RTGTRLREDYIWSVFIQSVKGLQVLHSL----------------------------N
Cnk1-Chlamydomonas            ---KLRTPFPEEAVWRIFLQLCKGLQALHSQ----------------------------N
Cnk2-Chlamydomonas            ---MMKRPLPEDMIWKYFIQVVMGLQALHSM----------------------------K
NRK11-T.thermophila           -----NQFINENKIWLFFLQMLLGLHSIHQQ----------------------------K
NRK17-T.thermophila           -----GRPLQEMKIWKYFIMSCMGLDYIHRK----------------------------K
NRK8-T.thermophila            -----GRPLVEKKIWKFFFQIAEGLLELHTR----------------------------N
FA2-Chlamydomonas             -----GRPLPEEGVWRIFIQTLIGLSYLHSK----------------------------K
Cnk4-Chlamydomonas            -----SR-LTEDLIWKLYIQILLGLNHMHSK----------------------------K
NEK1-H.sapiens                -----GVLFQEDQILDWFVQICLALKHVHDR----------------------------K
NEK3-H.sapiens                -----GKLFPEDMILNWFTQMCLGVNHIHKK----------------------------R
NRK26-T.thermophila           ---EKGQLFSEKQIIDWFVQMALAIKHVHDR----------------------------K
NRK29-T.thermophila           ---KTGQGFTEQQILEWFVQICFGLKFIHDR----------------------------R
NEK4-H.sapiens                -----GQLLPENQVVEWFVQIAMALQYLHEK----------------------------H
NRK22-T.termophila            -----GKNFPEDQIQDWLAQMALALFYLHEK----------------------------K
NRK10-T.thermophila           ---KENDHFPENIILNWFIQLTMALDFIHEK----------------------------H
NRK30-T.thermophila           ---AKGEQIEESIILQWFIQICSALSFIHSK----------------------------K
NRK13-T.thermophila           ---QKKEYFPEMLIVNWFYELALSIKYIHEK----------------------------K
Cnk5-Chlamydomonas            ---AAKEYFTEDEVMNMFVQIASAISYIHSK----------------------------R
NEK11-H.sapiens               ---QAGKIFPENQIIEWFIQLLLGVDYMHER----------------------------R
NRK19-T.thermophila           -----GKYFKEEQILDWFTQICLAMKHVHDR----------------------------K
NRK21-T.thermophila           -----GKYLQEKQILDWFTQICLAMKHVHDR----------------------------K
NRK12-T.thermophila           -----GKFIPENQILDWFTQICLALKHIHDR----------------------------K
NRK27-T.thermophila           -----SN-IPEEQILNWLLQMSLALNYVHNK----------------------------N
NRK15-T.thermophila           ---QIGKPFEEEQILDWFAQICQGLKYIHEN----------------------------K
NRK25-T.thermophila           ---QTGKLFEEDQILDWFIQICLGLKYIHKN----------------------------K
NRK14-T.thermophila           ---QSAKPFEEEKILDFFIQICLGLDCIHKN----------------------------K
NRK16-T.thermophila           ---TKGIRIDEETILYFTAQIVIALFFMHQK----------------------------K
NRK24-T.thermophila           ---QKGIPIDEETVLYFTAQIIISVLFMHSK----------------------------N
Cnk3-Chlamydomonas            ---AEKKPYSEDEIMFWFVQIVLALYHVHGR----------------------------N
Nrk-C.fasciculata             ---KEHLPFQEYEVGLLFYQIVLALDEVHTR----------------------------R
```

```
CAMKK-M.musculus             -------FYTERDASRLIFQVLDAVKYLHDL----------------------------G

NEK8-H.sapiens               ILHRDLKTQNILLDKHRMV--------------------------------------VKI
Nek8-2-C.elegans             IVHRDLKPQNILMNRRKTV--------------------------------------LKL
Nek8-1-C.elegans             VLHRDLKTANVFLTRDSF---------------------------------------VKI
Nek8-similar-D.melanogaster  ILHRDLKTANVFLNRRGI---------------------------------------VKI
NEK9-_Nercc1                 ILHRDIKTLNIFLTKANL---------------------------------------IKL
NRK18-T.thermophila          ILHRDLKPANIFLDAQNN---------------------------------------IKL
NRK20-T.thermophila          IMHRDLKPANIFLDSKNN---------------------------------------AKL
NEK2-H.sapiens               VLHRDLKPANVFLDGKQN---------------------------------------VKL
Nek2-similar-D.melanogaster  ILHRDIKPANIFLDAAGN---------------------------------------AKL
NIM-1-N.crassa]              ILHRDLKPENVFLGEDNS---------------------------------------VKL
NIMA-A.nidulans              ILHRDLKPENIFLGSDNT---------------------------------------VKL
KIN3-S.cerevisiae_           VIHRDLKPGNIFLSYDDSDYNINEQVDGHEEVNSNYYRDHRVNSGKRGSPMDYSQVVVKL
Fin1-S.pombe                 VLHRDIKPANIFLDENNS---------------------------------------VKL
NEK6-H.sapiens               VMHRDIKPANVFITATGV---------------------------------------VKL
NEK7-H.sapiens               VMHRDIKPANVFITATGV---------------------------------------VKL
Nek7-similar-C.elegans       IMHRDIKPANVFITGNGI---------------------------------------VKL
Cnk6-Chlamydomonas           IMHRDIKPANVLVGANGA---------------------------------------LKL
NRK1-T.thermophila           ILHRDLKGANVFIAEDGS---------------------------------------LRL
NRK3-T.thermophila           ILHRDLKCANIFLGANNK---------------------------------------VKL
NRK2-T.thermophila           ILHRDLKCANVFISKQDE---------------------------------------YKI
NRK-31-T.termophila          ILHRDLKCANVFLSLEGV---------------------------------------YKL
NRK4-T.thermophila           ILHRDFKCANIMLTRDHKN--------------------------------------LKL
NRK5-T.thermophila           ILHRDLKAANVFLDKYQTR--------------------------------------AML
NRK7-T.thermophila           ILHRDLKCANIFLDSRRN---------------------------------------AKI
NRK9-T.thermophila           IVHRDLKCANIFIGDNNI---------------------------------------IKI
NRK6-T.thermophila           IYHRDIKNANLFLYKDGT---------------------------------------TKI
Cnk1-Chlamydomonas           IIHRDIKPANIFLCANDL---------------------------------------LKI
Cnk2-Chlamydomonas           ILHRDIKPGNIMVFDNGV---------------------------------------AKI
NRK11-T.thermophila          VLHRDFKTMNIFLTKNSTE--------------------------------------IRI
NRK17-T.thermophila          ILHRDIKAMNIFLNKDDS---------------------------------------LRI
NRK8-T.thermophila           ILHRDIKTMNLFLTGNEQ---------------------------------------IRI
FA2-Chlamydomonas            IIHRDIKSANLFIDAYDN---------------------------------------IKI
Cnk4-Chlamydomonas           ILHRDIKTLNVFLDEDLN---------------------------------------VKL
NEK1-H.sapiens               ILHRDIKSQNIFLTKDGT---------------------------------------VQL
NEK3-H.sapiens               VLHRDIKSKNIFLTQNGK---------------------------------------VKL
NRK26-T.thermophila          ILHRDLKTQNIFLNAKGD---------------------------------------IKI
NRK29-T.thermophila          ILHRDLKTQNIFLTKSKQ---------------------------------------IKI
NEK4-H.sapiens               ILHRDLKTQNVFLTRTNI---------------------------------------IKV
NRK22-T.termophila           ILHRDLKTQNIFL-KSGR---------------------------------------VRL
NRK10-T.thermophila          VLHRDVKSSNIFLTSSGS---------------------------------------IKL
NRK30-T.thermophila          VIHRDIKSSNIFLTKSNC---------------------------------------VKI
NRK13-T.thermophila          ILHRDIKTSNIFITRDGT---------------------------------------IKI
Nrk5-Chlamydomonas           VLHRDLKTQNIFIAKGGI---------------------------------------IKL
NEK11-H.sapiens              ILHRDLKSKNVFL-KNNL---------------------------------------LKI
NRK19-T.thermophila          ILHRDLKGQNIFLTSQNI---------------------------------------CKL
NRK21-T.thermophila          IIHRDLKGQNIFLTRNHI---------------------------------------VKL
NRK12-T.thermophila          IVHRDLKTQNIFLMKDNA---------------------------------------LKV
NRK27-T.thermophila          IIHRDIKAQNVFLSANNI---------------------------------------VKL
NRK15-T.thermophila          IIHRDIKPANLFLTAQNT---------------------------------------VKI
NRK25-T.thermophila          IIHRDIKPENIFLTADNR---------------------------------------VKI
NRK14-T.thermophila          IIHRDIKPANIFLTAFGQ---------------------------------------LKI
NRK16-T.thermophila          ILHRDIKSQNLFLTKENV---------------------------------------VKL
NRK24-T.thermophila          ILHRDIKTQNLFLTKENI---------------------------------------VKL
Cnk3-Chlamydomonas           VLHRDLKSQNIFIAEGNL---------------------------------------LKL
Nrk-C.fasciculata            MMHRDLKSANIFLMPTGI---------------------------------------IKL
```

```
CAMKK-M.musculus              IVHRDLKPENLLYYSLDEDSK------------------------------------IMI


NEK8-H.sapiens                GDFGISKILSSKS-----KAYTVVGTPCYISPELCEGKPYNQKSDIWALGCVLYELASLK
Nek8-2-C.elegans              SDFGISKELGTKS-----AASTVIGTPNYLSPEICESRPYNQKSDMWSLGCVLYELLQLE
Nek8-1-C.elegans              GDFGISKIMGTETLAQ--GAKTVVGTPYYISPEMCSGVSYNEKSDMWALGCILYEMCCLK
Nek8-similar-D.melanogaster   GDFGISKIMNTKI-----HAQTVLGTPYYFSPEMCEGKEYDNKSDIWALGCILGEMCCLK
NEK9-_Nercc1                  GDYGLAKKLNSEYS----MAETLVGTPYYMSPELCQGVKYNFKSDIWAVGCVIFELLTLK
NRK18-T.thermophila           GDFGLSRVMGEQSE----FADTHVGTPYYMSPEQIQEKKYNEKSDIWSAGCLLYEMAALK
NRK20-T.thermophila           GDFGLSKKLSDETK----FAYTNVGTPYYMSPEQIEENKYNEKSDIWACGCLLYELGALS
NEK2-H.sapiens                GDFGLARILNHDTS----FAKTFVGTPYYMSPEQMNRMSYNEKSDIWSLGCLLYELCALM
Nek2-similar-D.melanogaster   GDFGLARMLRRDQS----FAASFVGTPHYMSPELVKGRKYDRKSDVWAVGCLVYEMCALR
NIM-1-N.crassa]               GDFGLSKVMQSHD-----FASTYVGTPFYMSPEICAAEKYTLKSDIWSLGCIIYELCARE
NIMA-A.nidulans               GDFGLSKLMHSHD-----FASTYVGTPFYMSPEICAAEKYTLRSDIWAVGCIMYELCQRE
KIN3-S.cerevisiae_            GDFGLAKSLETSIQ----FATTYVGTPYYMSPEVLMDQPYSPLSDIWSLGCVIFEMCSLH
Fin1-S.pombe                  GDFGLSKLLDNTRV----FTQSYVGTPYYMSPEIIRSSPYSAKSDVWALGCVIFEICMLT
NEK6-H.sapiens                GDLGLGRFFSSETT----AAHSLVGTPYYMSPERIHENGYNFKSDIWSLGCLLYEMAALQ
NEK7-H.sapiens                GDLGLGRFFSSKTT----AAHSLVGTPYYMSPERIHENGYNFKSDIWSLGCLLYEMAALQ
Nek7-similar-C.elegans        GDLGLGRFFSSKTT----AAHSLVGTPYYMSPERIQESGYNFKSDLWSTGCLLYEMAALQ
Cnk6-Chlamydomonas            GDLGLGRQLSEQTM----EAFSKVGTPYYVSPEVVRGAGYDWKSDVWSMGCLLYELACLR
NRK1-T.thermophila            GDLNVSKVQKRD------FAYTQTGTPYYTSPEVWQNRPYDSKCDVWSLGCVLYEIVTLE
NRK3-T.thermophila            GDLNVSKIMKRD------LAYTQTGTPYYTSPEVWQNQPYDSKCDVWSMGCVLYELMAHH
NRK2-T.thermophila            GDLNVSKVAKRG------LVYTQTGTPYYASPEVWRDEPYDSSSDIWSFGCILYELAALN
NRK-31-T.termophila           GDLNVSKVAKKG------LVYTQTGTPYYASPEVWRDEPYDVKSDIWSLGCVLYEMCALK
NRK4-T.thermophila            GDLNVSKVAKKG------MLYTQTGTPYYASPEVWRDKPYNSKSDVWSLGCVIYELVSLN
NRK5-T.thermophila            GDLNVSKKLQVNG-----LLYTQTGTPYYASPEVWKDKPYNNKSDIWSLGCVIYEMCALK
NRK7-T.thermophila            GDLNVSKITQAN------LARTQVGTPYYTSPEVWKDQMYNNKSDIWSLGCLIYELCAQK
NRK9-T.thermophila            GDLNVSKVTRRPEQ----MAKTQTGTPYYTAPEVWKGLSYNYKSDVWSLGCIIYELSAQK
NRK6-T.thermophila            GDFNVSKQAKMG------LLYTQTGTPYYASPEVWSDQPYTSSSDIWSLGCVLYELATLS
Cnk1-Chlamydomonas            GDLGIAKALTSMN-----FARTQIGTPCYMAPEVWSGRPYSYSSDMWSLGAVLYEMMTFR
Cnk2-Chlamydomonas            GDLGIAKLLTKTA-----AAKTQIGTPHYMGPEIWKNRPYSYTSDTWAIGCLLYELAALA
NRK11-T.thermophila           GDLGVAKYLGDTNN----LAKTMVGTPYYLSPEICEEKPYNEKSDIWSLGCILYELCTFK
NRK17-T.thermophila           GDLGVAKVLSDQGN----FASTMVGTPFYLSPEMCEEKPYNEKSDIWSLGCVLYELCTYR
NRK8-T.thermophila            GDLGVAKQLENNKS----HAHSQVGTPYYMSPEIIQDIPYNEKSDVWSLGCVLYQLATFK
FA2-Chlamydomonas             GDFGIARSLGASSN----LAQTILGTPYYMAPELCQDKPYDAKSDVWALGVVMYECCMGH
Cnk4-Chlamydomonas            GDMGVAKILSTNTV----FAKTIVGTPYYLSPELCEDKPYNEKSDVWALGVVLYECCTQR
NEK1-H.sapiens                GDFGIARVLNSTVE----LARTCIGTPYYLSPEICENKPYNNKSDIWALGCVLYELCTLK
NEK3-H.sapiens                GDFGSARLLSNPMA----FACTYVGTPYYVPPEIWENLPYNNKSDIWSLGCILYELCTLK
NRK26-T.thermophila           GDFGIARVLQHTYD----CAKTAIGTPYYLSPEICQEKPYNQKSDIWSLGCILYEMTTLN
NRK29-T.thermophila           GDFGI--VLQNTCE----MAKTAIGTPYYLSPEICQ--PYNQKTDIWSLGCILYELCTLR
NEK4-H.sapiens                GDLGIARVLENHCD----MASTLIGTPYYMSPELFSNKPYNYKSDVWALGCCVYEMATLK
NRK22-T.termophila            GDFGIAKVLDSTRD----FANTCIGTPYYMSPELFKYKPYSYKSDIWAFGCVLYEMCNLR
NRK10-T.thermophila           GDFGISKVLHSTAD----KAQTLIGTPYYLSPEVCENKPYTYQSDIWALGCVLFEMCALK
NRK30-T.thermophila           GDFGISKQLENSMD----KANTLVGTPYYLSPEVCQNKPYTYKSDMWSLGCVIYELCSLK
NRK13-T.thermophila           GDFGISKVLENTTS----VANTVVGTPYYMSPEVCESKPYTYKSDIWALGCVLYELCALE
Cnk5-Chlamydomonas            GDFGISKVLERTDS----FATTVTGTPYYMAPEICTNQPYTYKSDIWSLGCVLYELCTLR
NEK11-H.sapiens               GDFGVSRLLMGSCD----LATTLTGTPHYMSPEALKHQGYDTKSDIWSLACILYEMCCMN
NRK19-T.thermophila           GDFGIARVLNKTFE----KAKTMVGTPYYLSPEIINSVPYSYKSDVWSIGVVLYEMCCLR
NRK21-T.thermophila           GDFGIARVLSKTVE----KAKTMVGTPYYLSPEIIESKPYSFKTDIWSLGVILYELCALK
NRK12-T.thermophila           GDFGIAKVLRHTRE----NCKTMVGTPYYISPEILEAKPYSFRSDIWSLGLFIY-MCAQK
NRK27-T.thermophila           ADFGIARILSCTKD----KAQTFIGTPYYLAPELVNSDPYTTKADIWSLGVLIYHLCALK
NRK15-T.thermophila           GDFGVAKEQKYTNE----QLSSQIGTPYYLAPEIIDGKPYNSSVDIWSLGVVLYELCCLK
NRK25-T.thermophila           GDFGISKELEYTNQ----QLYSRIGTPYYLAPEMIKGELYNSSVDIWSLGVVLYELCCLK
NRK14-T.thermophila           GDFGVSKELQYTKQ----CLKTIVGTPYYIAPEIVSGYAYNSSVDIWSLGVILYQLCCLQ
NRK16-T.thermophila           GDFGISKALGTNAD----FTKTLVGTPYFMSPEVCAGQSYGDKADIWALGCTLYEMVMLR
NRK24-T.thermophila           GDFGISKELGTNAN-----AKTLVGTPYFMSPEVCSGENYGQKADIWAIGCTLYEMVMLK
Cnk3-Chlamydomonas            GDFGIARVLNSDTE----LARTVIGSPYYLSPEICEDRPYNRKSDVWSLGCVLYELATLR
Nrk-C.fasciculata             GDFGFSKQYNDSVSLD--VGSSFCGTPYYLAPELWERKRYSKKADMWSLGVILYELLTLH
```

CAMKK-M.musculus	SDFGLSKMEDPGS-----VLSTACGTPGYVAPEVLAQKPYSKAVDCWSIGVIAYILLCGY
NEK8-H.sapiens	RAFEAA---NLPALVLKIMSGTFAPIS--DRYSPELRQLVLSLLSLEPAQ---RPPLSHI
Nek8-2-C.elegans	RAFDGE---NLPAIVMKITRSKQNPLG--DHVSNDVKMLVENLLKTHTDK---RPDVSQL
Nek8-1-C.elegans	KAFEGD---NLPALVNSIMTCAYTPVK--GPYSAEMKMVIRELLQLDPQK---RPSAPQA
Nek8-similar-D.melanogaster	KTFAAS---NLSELVTKIMAGNYTPVP--SGYTSGLRSLMSNLLQVEAPR---RPTASEV
NEK9-_Nercc1	RTFDAT---NPLNLCVKIVQGIRAMEVDSSQYSLELIQMVHSCLDQDPEQ---RPTADEL
NRK18-T.thermophila	PPFEAT---NHLSLAIKIKSGKFERLP--LRYSEELQKLIESMVHIDPEK---RPSVQNI
NRK20-T.thermophila	PPFPAT---NHLALAMKIKNGKFERLS--KQYSDELMRVISWCLQKNSEN---RPSVDDL
NEK2-H.sapiens	PPFTAF---SQKELAGKIREGKFRRIP--YRYSDELNEIITRMLNLKDYH---RPSVEEI
Nek2-similar-D.melanogaster	PPFRGR---AFDQLSEKIAQGEFSRIP--AIYSTDLQEIIAFMLAVDHEQ---RPGIEVI
NIM-1-N.crassa]	PPFNAK---THYQLVQKIKEGKIAPLP--SVYSGELFATIKDCLRVNPDR---RPDTATL
NIMA-A.nidulans	PPFNAR---THIQLVQKIREGKFAPLP--DFYSSELKNVIASCLRVNPDH---RPDTATL
KIN3-S.cerevisiae_	PPFQAK---NYLELQTKIKNGKCDTVP--EYYSRGLNAIIHSMIDVNLRT---RPSTFEL
Fin1-S.pombe	HPFEGR---SYLELQRNICQGNLSCWD--HHYSDDVFLLIRHCLEVNSDL---RPTTYQL
NEK6-H.sapiens	SPFYGDKM-NLFSLCQKIEQCDYPPLPG-EHYSEKLRELVSMCICPDPHQ---RPDIGYV
NEK7-H.sapiens	SPFYGDKM-NLYSLCKKIEQCDYPPLPS-DHYSEELRQLVNMCINPDPEK---RPDVTYV
Nek7-similar-C.elegans	SPFYGDKM-NLYSLCKKIENCEYPPLPA-DIYSTQLRDLVSRCILPEASK---RPETSEV
Cnk6-Chlamydomonas	SPFEMEGA-NLYDVFQKISKGEYSPLPA-DQFSAPLRSLVGRMLQIDPAK---RPELEEV
NRK1-T.thermophila	PPFKGT---SMEDLYKRVLRGNFSPINL-QRYSSDIQKFIESCLKVEPKM---RSSVESL
NRK3-T.thermophila	PPFEAK---SMEELYKKVCKGTYQKLP--KQYSQEMNDFINLCLRKNPKQ---RPSVNSL
NRK2-T.thermophila	PPFRAK---DMEGLYKKVQKGIFERIP--QRYSNDLQKFIALCLQVSSVQ---RPSVTQL
NRK-31-T.termophila	PPFRAN---DMEGLYKKVQKGDFERIP--KKYSEDLQRMLTMLLKVNPKD---RPSCEQI
NRK4-T.thermophila	PPFKAQ---DMEGLFKKVQKGQYDPIP--SWYSQDLTDFLSLCLQVNPKM---RLTTSEL
NRK5-T.thermophila	PPFKGK---DMEDLFKKVQRGVYDPIP--SHFSKELNLFIAQLLRVNPEQ---RPNCDEI
NRK7-T.thermophila	PPFLAS---DMPSLFKKIGKGIYERIP--SRYSSELSNLISQCLNINQIT---RPDCDQL
NRK9-T.thermophila	HPFKGN---TIEGLFTNIMKGQYERIP--SFYSEELAFVISQCLLQNPKL---RPGCDQL
NRK6-T.thermophila	PPFNGE---SMKELYGKIMSGVYKKIS--PKYSLELSNMVSNMLQIDPKK---RFSCSQI
Nrk1-Chlamydomonas	TPMEGR---TMADLRNRIKGGRYTPIPA-GRYSAELTNICHSLLATDPAK---RPSPTSI
Cnk2-Chlamydomonas	VPFEAR---SMSELRYKVLRGTYPPIP--NTFSRDLQQMVRECLDPNPDK---RPTMDQI
NRK11-T.thermophila	HPFEAS---NQGALVIKILKNKVEPLP--SMYSRELQSIISLLLTKDHQK---RPDTTTL
NRK17-T.thermophila	HPFEAQ---NQGALVLRIIRGKYNPIP--TSYSKDLVSMVDMCLCKDYKK---RPNARDI
NRK8-T.thermophila	RPFEAT---NQGSLVLKIQKAQYIPIS--SNYSPQLHRLIELCLTKEHQK---RYSIKQL
FA2-Chlamydomonas	YPFDVENN-NQVALIRKIARGVFKPVS--GPYTQQLIQLITSCLTLDPRQ---RPDTTAL
Cnk4-Chlamydomonas	HPFDAD---NQGALILKILRGKFPPVS--G-YSPDICDLIKRCLTQNANR---RPNTYKL
NEK1-H.sapiens	HAFEAG---SMKNLVLKIISGSFPPVS--LHYSYDLRSLVSQLFKRNPRD---RPSVNSI
NEK3-H.sapiens	HPFQAN---SWKNLILKVCQGCISPLP--SHYSYELQFLVKQMFKRNPSH---RPSATTL
NRK26-T.thermophila	HAFDAN---SMKGLVLKILRGTYPPIP--EQYSQDLRDLISEMLIKDPTQ---RPSIRKI
NRK29-T.thermophila	HAFDAK---HQQGLVLKILKGNYPSIP--NCYSPQLSDLIGEMLQRHPAK---RPSVKKI
NEK4-H.sapiens	HAFNAK---DMNSLVYRIIEGKLPAMP--RDYSPELAELIRTMLSKRPEE---RPSVRSI
NRK22-T.termophila	HAFDAQ---SLNGLAVKIMNGTYPPIN--STYSKSLRDLIGKMLQLNPKS---RPSILDI
NRK10-T.thermophila	HPFVSE---SLMALVVKIIREPNPNIP--NMYSSDLNCLVNILLAKKPES---RPRTKQI
NRK30-T.thermophila	HPFQSN---SLMSLVMKIATEKAPKIP--QNYSLMTNGFIRSLLQKIPEK---RPSAQDI
NRK13-T.thermophila	HAFESN---NLLGLIFKIVQQNISDIP--SFYSKELNDLIQKLLNKNEQE---RPVINDI
Cnk5-Chlamydomonas	HAFAAD---SLLSLVYQIVRGNFPPIPT-DQFSNGLSDLVNRLLWRDPAT---RPSLSEV
NEK11-H.sapiens	HAFAGS---NFLSIVLKIVEGDTPSLP--ERYPKELNAIMESMLNKNPSL---RPSAIEI
NRK19-T.thermophila	PPFQGE---SLQNLALNIVKGQYQPIP--NIYSQDLKKLVSNLLQNRPES---RYSIQQI
NRK21-T.thermophila	PPFNAD---SLHFLALKIVKGQYSPIP--THFSKEMKNLVSNLLQVDAIR---RPSINDI
NRK12-T.thermophila	PPFDGI---GLSNLALKIVKGVYAPIS--SYYSKDMTQLISLLLNVDPSK---RPQVNQI
NRK27-T.thermophila	PPFEAD---NIPSLMIKISRGQFPPIP--QIYSKELRYLVNEMLTVDHNQ---RPSALQI
NRK15-T.thermophila	PPFNSW---CEAGLYYKSKNLPYPPIQ--AKYSKKLKELISKLLRKSPDD---RYTLQEI
NRK25-T.thermophila	PPFNSW---NEYIIYYKTYKKPYPPITQ-AQYSPQLKTLISKLLIKSPEGERGRYTLEQI
NRK14-T.thermophila	PPFQDW---NEANLYCKIKKKPYPPIAQ-LYYSGKLKTLISKLLRKCPKE---RYTLKQI
NRK16-T.thermophila	RPFDCE---NINTLFTMIRQQDPSPLH--DNCSTDIRMLITLMLNKDPLK---RPFIWDL
NRK24-T.thermophila	RPFDND---NLNILFNKIRFEAPPPLH--ENTSTEIRMLITFMLQKDPVK---RPSVWDL
Cnk3-Chlamydomonas	RAFDGQ---SLPALVVKILRGKYPPVP--TRYSTPLRGLIESMLKQNPKD---RPSADAI
Nrk-C.fasciculata	RPFKGP---SQREIMQQVLYGKYDPFP--CPVSASMKALLDPLLSKDPED---RPTTQQL

```
CAMKK-M.musculus             PPFYDENDAKLFEQILKAEYEFDSPYW--DDISDSAKDFIRHLMEKDPEK---RFTCEQA


NEK8-H.sapiens               MAQPLCI------------------------
Nek8-2-C.elegans             LSDPLV-------------------------
Nek8-1-C.elegans             LKML---------------------------
Nek8-similar-D.melanogaster  LVYWI--------------------------
NEK9-_Nercc1                 LDRPLL-------------------------
NRK18-T.thermophila          LELPQI-------------------------
NRK20-T.thermophila          LNLPQI-------------------------
NEK2-H.sapiens               LENPLI-------------------------
Nek2-similar-D.melanogaster  IRHPLV-------------------------
NIM-1-N.crassa]              LNLPIV-------------------------
NIMA-A.nidulans              INTPVI-------------------------
KIN3-S.cerevisiae_           LQDIQIRTAR---------------------
Fin1-S.pombe                 LRSPIL-------------------------
NEK6-H.sapiens               HQVAKQMHIWM--------------------
NEK7-H.sapiens               YDVAKRMHAC---------------------
Nek7-similar-C.elegans       LQVAEHMNNYF--------------------
Cnk6-Chlamydomonas           WTI----------------------------
NRK1-T.thermophila           LNHKCIM------------------------
NRK3-T.thermophila           LEFQGLL------------------------
NRK2-T.thermophila           LNNVLL-------------------------
NRK-31-T.termophila          LSNPIVQRNGGE-------------------
NRK4-T.thermophila           LETPY--------------------------
NRK5-T.thermophila           LKFSVI-------------------------
NRK7-T.thermophila           LNLPII-------------------------
NRK9-T.thermophila           LKLP---------------------------
NRK6-T.thermophila           LQLPYKI------------------------
Cnk1-Chlamydomonas           L------------------------------
Cnk2-Chlamydomonas           L------------------------------
NRK11-T.thermophila          LSNSSN--IL---------------------
NRK17-T.thermophila          LQTQSNYSIL---------------------
NRK8-T.thermophila           LTDPGK-------------------------
FA2-Chlamydomonas            L------------------------------
Nrk4-Chlamydomonas           L------------------------------
NEK1-H.sapiens               LEKGFI-------------------------
NEK3-H.sapiens               LSRGIV-------------------------
NRK26-T.thermophila          LEKDFL-------------------------
NRK29-T.thermophila          LEKQFL-------------------------
NEK4-H.sapiens               LRQPYI-------------------------
NRK22-T.termophila           INTSFV-------------------------
NRK10-T.thermophila          LSFPFV-------------------------
NRK30-T.thermophila          LNNQNI-------------------------
NRK13-T.thermophila          LNDI---------------------------
Cnk5-Chlamydomonas           FKL----------------------------
NEK11-H.sapiens              LKIPYL-------------------------
NRK19-T.thermophila          LGLPFI-------------------------
NRK21-T.thermophila          MKMPII-------------------------
NRK12-T.thermophila          LKLPII-------------------------
NRK27-T.thermophila          LGRFF--------------------------
NRK15-T.thermophila          LDEPFI-------------------------
NRK25-T.thermophila          LNEPLI-------------------------
NRK14-T.thermophila          FDESLI-------------------------
NRK16-T.thermophila          VNIPII-------------------------
NRK24-T.thermophila          AKIPII-------------------------
Cnk3-Chlamydomonas           LKSDFV-------------------------
Nrk-C.fasciculata            LQTEFM-------------------------
```

```
CAMKK-M.musculus              LQHPWI-------------------------
```