Supplementary Data to
Structural determinants of discrimination of NAD^+ from NADH in yeast mitochondrial NADH kinase Pos5 [*]

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Supplementary Figure legends

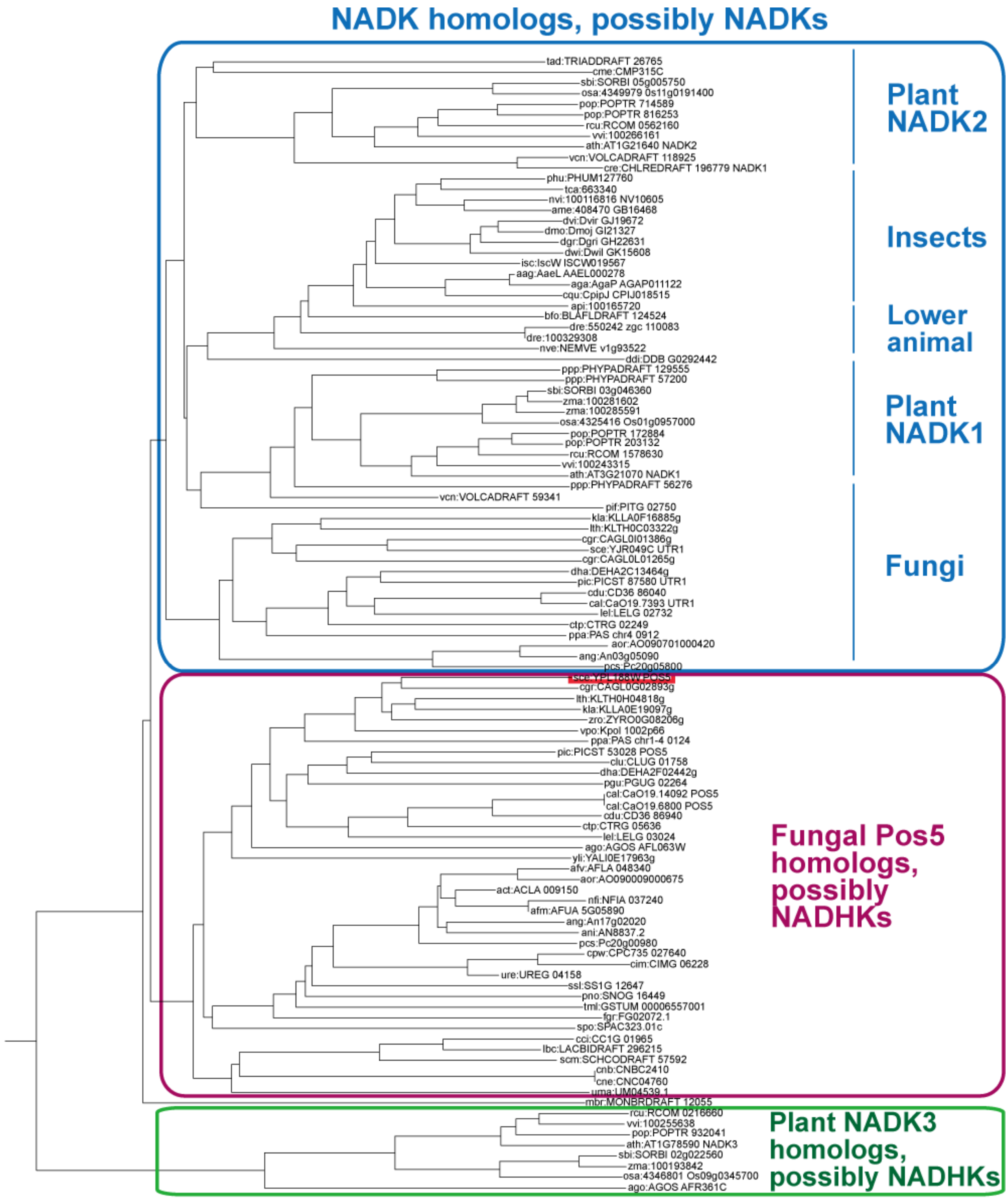
Fig. S1. Phylogenetic tree constructed using the 100 proteins most homologous with Pos5, and also the NADK3 homologs including NADK3 (8 proteins). Pos5 is boxed in red.

Fig. S2. (A) The stereo view of the conformation of NADH in Pos5 and NAD^{+} in NADKs whose tertiary structures have been solved. NAD^{+} molecules are overlaid just on the NADH molecule by Pymol program using "align" command. The NADH in Pos5 is in blue; the NAD^{+} in Ppnk (PDB: 1u0t) (23) is in salmon pink, while those in *Listeria monocytogenes* NADK (2q5f) (25) and *Archaeoglobus fulgidus* NADK (1suw) (24) are black and yellow, respectively. (B, C) Stereo views of the ribbon models of Pos5–NADH-A/-B (B) and Pos5–NADH-A/-A' (C) in Fig. 1C.

Fig. S3. The Pos5-specific structures. (A) Stereo view of the Cα backbone traces of superimposed structures of Pos5 (thin blue) and Ppnk–NAD^{+} (pink). The three additional Pos5-specific structures (c1', c2, and c3) comprising 27–65 (Leu-27 to Asn-65), 316–320 (Leu-316 to Pro-320), and 350–377 (Gly-350 to Arg-377) residues are emphasized in blue. (B) Multiple alignment of N-terminal sequences of Pos5 homologs exhibiting homology with the c1' structure. Cgr_NK, Lth_NK, Kla_NK, Zro_NK, Vpo_NK, and Ppa_NK indicate N-terminal sequences of Pos5 homologs from *Candida glabrata*, *Lachancea thermotolerans*, *Kluyveromyces lactis*, *Zygosaccharomyces rouxii*, *Vanderwaltozyma polyspora*, and *Pichia pastoris* belonging to family of *Saccharomycetes*. The c1' structure is indicated with blue arrows. Multiple alignment was conducted using ClustalW (21). The mitochondria-targeting sequences that were predicted using TargetP (22) are colored in green. The numbers of residues in each sequence are indicated. Identical and similar residues are marked with asterisks and dots, respectively.

Fig. S4. Multiple alignment of the primary structures of NADK3, Pos5 homologs, and NADK homologs including NADKs from human, *Methanococcus jannaschii*, *Mycobacterium tuberculosis*, *Escherichia coli*, and *Sphingomonas* sp. A1. Aligned residues corresponding to His-231, Thr-254, Thr-267, Ala-268, Ser-272, Cys-291, and Arg-293 are colored as shown.

Fig. S5. Electric charge on the molecular surfaces of NAD(H)-binding sites of other NADKs [Af_NK (1z0zA), Lm_NK (2i29A),Tm_NK (1yt5A), and St_NK (2an1B) from *A. fulgidus*, *L. monocytogenes*, *T. maritima*, and *S. typhimurim*, respectively.]. Positive and negative charges at pH 8.0 are colored in blue and red, respectively. NAD^{+} are colored as in Fig. 1A. Residues are colored in blue (His, Lys and Arg), red (Asp and Glu), yellow (Tyr, Ala, Gln, Pro, Ile, Phe, and Ser). The residues denoted by asterisks come from another subunit in each structure.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pop:POPTR 816253
rcu:RCOM 0562160
vvi:100266161
ath:AT1G21640 NADK2
vcn:VOLCADRAFT 118925
cre:CHLREDRAFT 196779 NADK1
phu:PHUM127760
tca:663340
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isc:IscW ISCW019567
aag:AaeL AAEL000278
aga:AgaP AGAP011122
cqu:CpipJ CPIJ018515
api:100165720
bfo:BLAFLDRAFT 124524
dre:550242 zgc 110083
dre:100329308
nve:NEMVE v1g93522
ddi:DDB G0292442
ppp:PHYPADRAFT 129555
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sbi:SORBI 03g046360
zma:100281602
zma:100285591
osa:4325416 Os01g0957000
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pop:POPTR 203132
rcu:RCOM 1578630
vvi:100243315
ath:AT3G21070 NADK1
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vcn:VOLCADRAFT 59341
pif:PITG 02750
kla:KLLA0F16885g
lth:KLTH0C03322g
cgr:CAGL0I01386g
sce:YJR049C UTR1
cgr:CAGL0L01265g
dha:DEHA2C13464g
pic:PICST 87580 UTR1
cdu:CD36 86040
cal:CaO19.7393 UTR1
lel:LELG 02732
ctp:CTRG 02249
ppa:PAS chr4 0912
aor:AO090701000420
ang:An03g05090
pcs:Pc20g05800
Plant NADK2
Insects
Lower animal
Plant NADK1
Fungi
sce:YPL188W POS5
cgr:CAGL0G02893g
lth:KLTH0H04818g
kla:KLLA0E19097g
zro:ZYRO0G08206g
vpo:Kpol 1002p66
ppa:PAS chr1-4 0124
pic:PICST 53028 POS5
clu:CLUG 01758
dha:DEHA2F02442g
pgu:PGUG 02264
cal:CaO19.14092 POS5
cal:CaO19.6800 POS5
cdu:CD36 86940
ctp:CTRG 05636
lel:LELG 03024
ago:AGOS AFL063W
yli:YALI0E17963g
afv:AFLA 048340
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nfi:NFIA 037240
afm:AFUA 5G05890
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pcs:Pc20g00980
cpw:CPC735 027640
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ssl:SS1G 12647
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fgr:FG02072.1
spo:SPAC323.01c
cci:CC1G 01965
lbc:LACBIDRAFT 296215
scm:SCHCODRAFT 57592
cnb:CNBC2410
cne:CNC04760
uma:UM04539.1
Fungal Pos5 homologs, possibly NADHKs
mbr:MONBRDRAFT 12055
rcu:RCOM 0216660
vvi:100255638
pop:POPTR 932041
ath:AT1G78590 NADK3
sbi:SORBI 02g022560
zma:100193842
osa:4346801 Os09g0345700
ago:AGOS AFR361C
Plant NADK3 homologs, possibly NADHKs

Fig. S1.

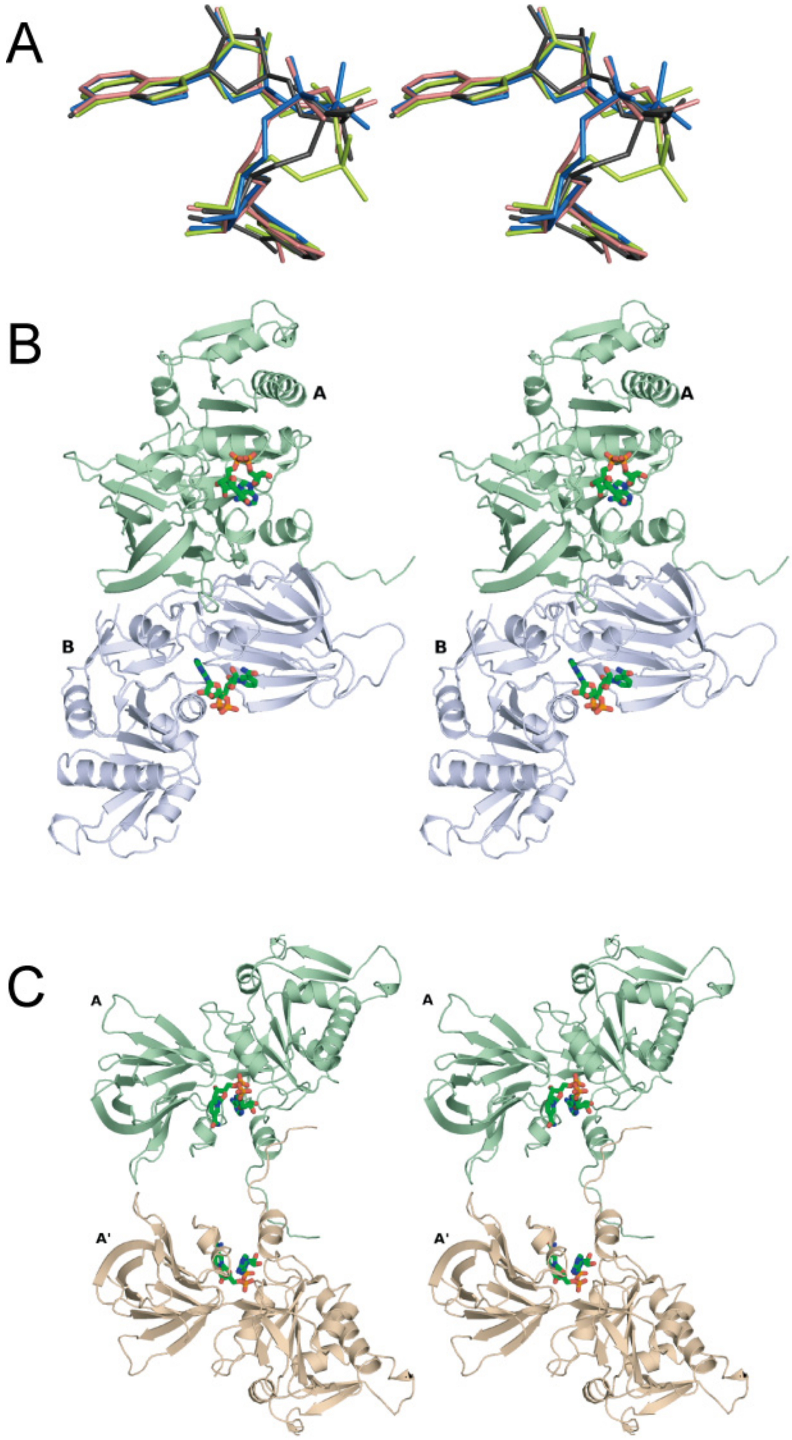
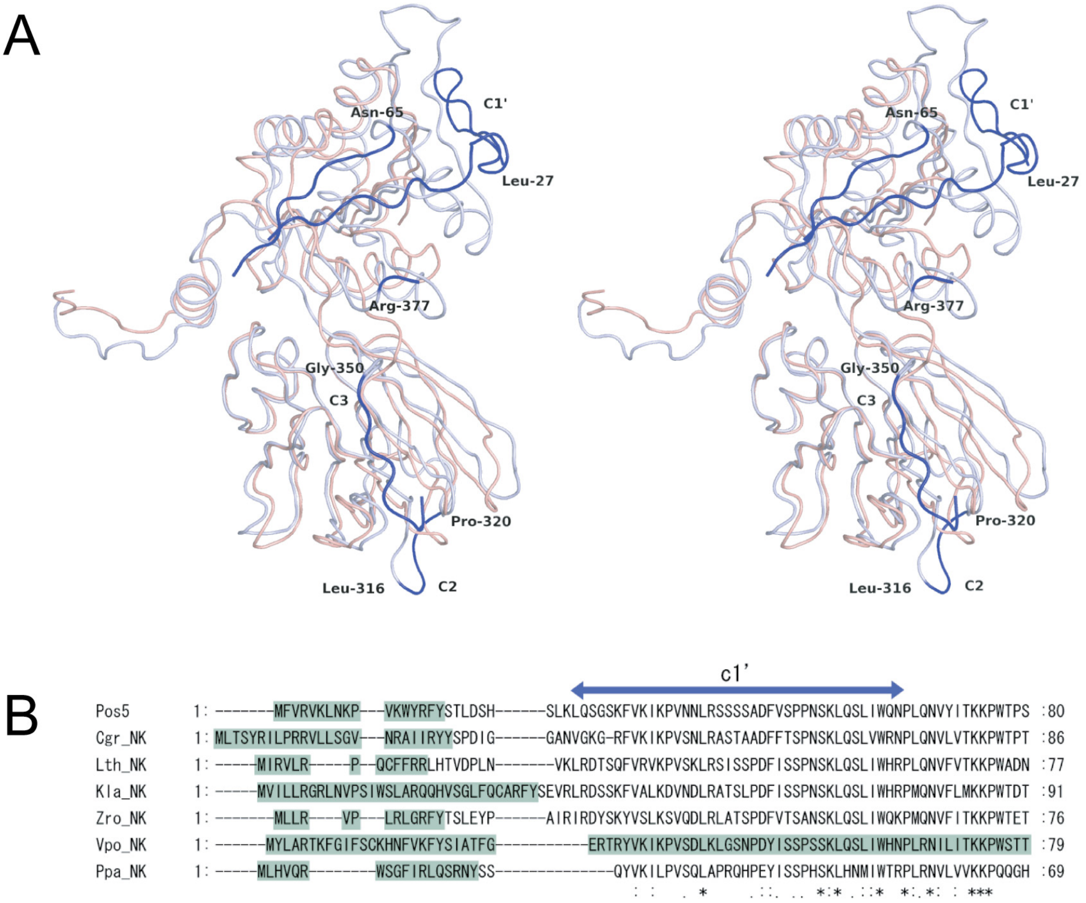


Fig. S2.



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Pos5     1: -------MFVRVKLNKP---VKWYRFYSTLDSH------SLKLQSGSKFVKIKPVNNLRSSSSADFVSPPNSKLQSLIWQNPLQNVYITKKPWTPS :80
Cgr_NK   1: MLTSYRILPRRVLLSGV---NRAIIRYYSPDIG------GANVGKG-RFVKIKPVSNLRASTAADFFTSPNSKLQSLVWRNPLQNVLVTKKPWTPT :86
Lth_NK   1: -----MIRVLR-----P--QCFFRRLHTVDPLN-------VKLRDTSQFVRVKPVSKLRSISSPDFISSPNSKLQSLIWHRPLQNVFVTKKPWADN :77
Kla_NK   1: -----MVILLRGRLNVPSIWSLARQQHVSGLFQCARFYSEVRLRDSSKFVALKDVNDLRATSLPDFISSPNSKLQSLIWHRPMQNVFLMKKPWTDT :91
Zro_NK   1: -------MLLR----VP---LRLGRFYTSLEYP------AIRIRDYSKYVSLKSVQDLRLATSPDFVTSANSKLQSLIWQKPMQNVFITKKPWTET :76
Vpo_NK   1: ------MYLARTKFGIFSCKHNFVKFYSIATFG-----------ERTRYVKIKPVSDLKLGSNPDYISSPSSKLQSLIWHNPLRNILITKKPWSTT :79
Ppa_NK   1: -----MLHVQR--------WSGFIRLQSRNYSS--------------QYVKILPVSQLAPRQHPEYISSPHSKLHNMIWTRPLRNVLVVKKPQQGH :69
                                                            : :   . *     .::. .. *:* .::*  *:.*: : ***
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Fig. S3.

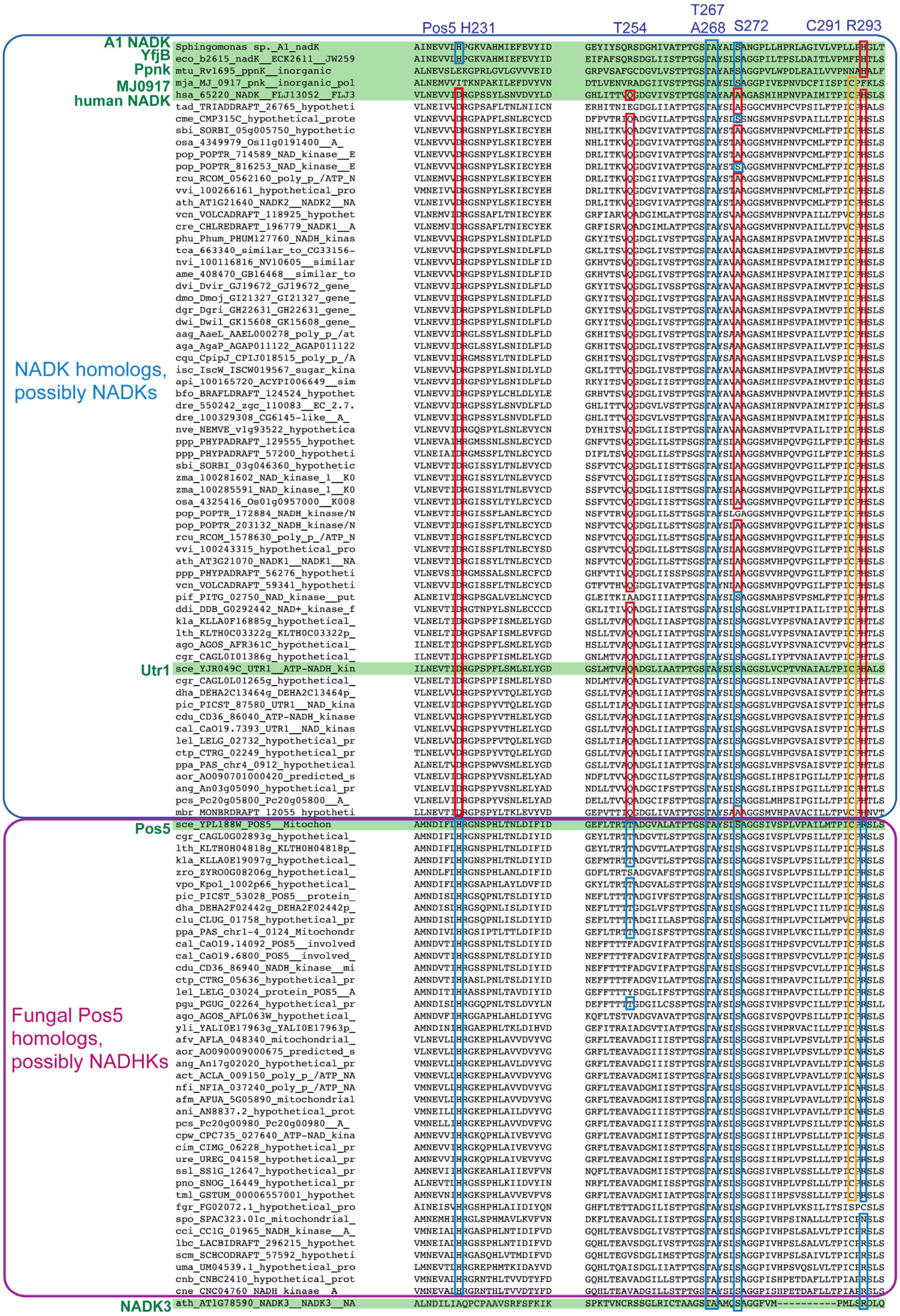
Pos5 H231
T254
T267
A268
S272
C291 R293
A1 NADK
YfjB
Ppnk
MJ0917
human NADK
NADK homologs, possibly NADKs
Utr1
Pos5
Fungal Pos5 homologs, possibly NADHKs
NADK3

Fig. S4.

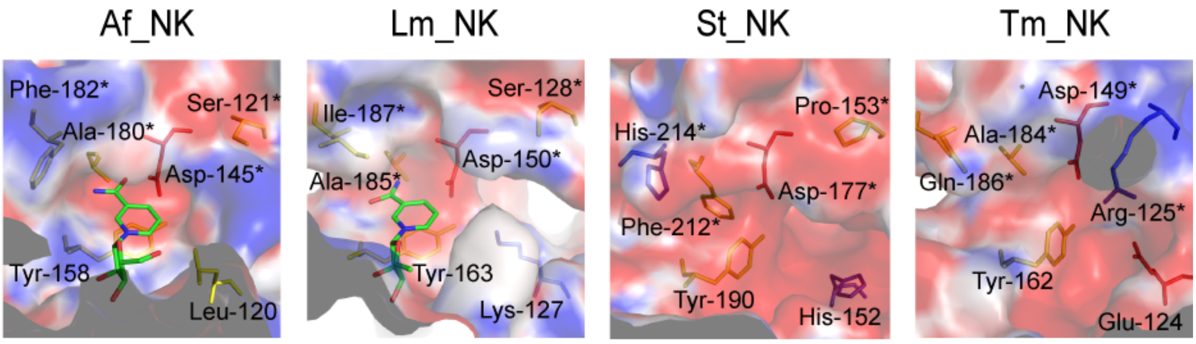
Af_NK
Phe-182*
Ser-121*
Ala-180*
Asp-145*
Tyr-158
Leu-120
Lm_NK
Ser-128*
Ile-187*
Asp-150*
Ala-185*
Tyr-163
Lys-127
St_NK
Pro-153*
His-214*
Asp-177*
Phe-212*
Tyr-190
His-152
Tm_NK
Asp-149*
Ala-184*
Gln-186*
Arg-125*
Tyr-162
Glu-124

Fig. S5.