## Supplementary Data to Structural determinants of discrimination of NAD<sup>+</sup> 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<sup>+</sup> in NADKs whose tertiary structures have been solved. NAD<sup>+</sup> molecules are overlaid just on the NADH molecule by Pymol program using "align" command. The NADH in Pos5 is in blue; the NAD<sup>+</sup> 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<sup>+</sup> (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<sup>+</sup> 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.



# NADK homologs, possibly NADKs

Fig. S1.



Fig. S2.





		Post	Pos5 H231		T: 54 A:	267 268 ;	S272 C291 F	R293
A1 NADK	Sphingomonas spAl_nadK	AINEVVI	HPGKVAHMIEFEVYID	GEYIYSQR	SDGMIVATPTGS	FAYAL	SANGPLLHPRLAGIVLVPL	LFHGLT
Ppnk	eco_b2615_nadKECK2611JW259 mtu Rv1695 ppnK inorganic	AINEVVI ALNEVSI	HPGKVAHMIEFEVYID EKGPRLGVLGVVVEID	EIFAFSQR GRPVSAFG	SDGLIISTPTGS CDGVLVSTPTGS	FAYSI FAYAF	SAGGPILTPSLDAITLVPM SAGGPVLWPDLEAILVVPM	IFFHTLS
MJ0917	mja_MJ_0917_pnk_inorganic_pol	ALNEMV	ITKNPAKILEFDVYVN	DTLVENVR	ADGIIVSTPTG	TAYSI	SAGGPIVEPNVDCFIISPI	CPFKLS
human NADK	tad_TRIADDRAFT_26765_hypotheti	VLNEIV	DRGPSAFLTNLNIICN	ERHITNIE	GDGVIVSTPTGS	TATAA TAYSI	ASGGCMVHPCVPSILFTPI	C HALS
	cme_CMP315C_hypothetical_prote sbi SORBI 05g005750 hypothetic	VLNEVV	DRGPAPFLSNLECYCD DRGSNPYLSKIECYEH	DFPVTRIQ NHLITKVO	ADGVILATPTGS ADGVIVATPTGS	FAYSI FAYSI	SSNGSMVHPSVPAILLTPIC AAGGSMVHPNVPCMLFTPIC	C PHSLS
	osa_4349979_0s11g0191400A_	VLNEVVV	DRGSNPYLSKIECYEH	NHLITKVQ	GDGVIVATPTGS	FAYST	AAGGSMVHPNVPCMLFTPI	CHESLS
	pop_POPTR_714589_NAD_kinase_E pop_POPTR_816253_NAD_kinase_E	VLNEVV	DRGSNPYLSKIECYEH	DRLITKVQ	GDGVIVATPTGS GDGVIVATPTGS	FAYST.	AGGSMVHPNVPCMLFTPI	CHESLS
	rcu_RCOM_0562160_poly_p_/ATP_N	VLNEMV	DRGSNPYLSKIECYEH	DRLITKVQ	GDGIIVATPTGS	TAYST	AGGSMVHPNVPCMLFTPI	CPHSLS
	vvi_100266161_hypothetical_pro ath AT1G21640 NADK2 NADK2 NA	VMNEIV	DRGSNPYLSKIECYEH	DRLITKVQ	GDGVIVATPTGS GDGVIVATPTGS	FAYST.	AGGSMVHPNVPCMLFTPI	CHESLS
	vcn_VOLCADRAFT_118925_hypothet	VLNEMVI	DRGSSAFLTNIECYEK	GRFIARVQ	ADGIMLATPTGS	FAYSU	AAGGSMVHPNVPAILLTPV	CHESLS
	cre_CHLREDRAFT_196779_NADK1A	VLNEMVI	DRGSSAFLTNIECYEK	GRFISRVQ	ADGIMLATPTGS CDGLIVSTPTGS	FAYSV.	AAGGSMVHPNVPAILLTPV AAGASMTHPSVPAIMVTPT	CHSLS
	tca_663340_similar_to_CG33156-	VLNEVV	DRGPSPYLSNIDLFLD	GKLITSVQ	GDGLIVSTPTGS	FAYAU	AAGASMIHPSVPAIMVTPI	CHSLS
	nvi_100116816_NV10605similar ame 408470 GB16468_similar to	VLNEVV	DRGPSPYLSNIDLFID	GKHVTSVQ	GDGLIVSTPTGS GDGLIVSTPTGS	FAYAU	AAGASMIHPSVPAIMITPIC AAGASMIHPSVPAIMITPIC	CHESLS
	dvi_Dvir_GJ19672_GJ19672_gene_	VLNEVVI	DRGPSPYLSNIDLFLD	GKYITSVQ	GDGLIVSSPTGS	FAYAV	AGASMIHPSVPAIMVTPI	CHSLS
	dmo_Dmoj_GI21327_GI21327_gene_ dar Dari GH22631_GH22631_gene	VLNEVVI VLNEVVI	DRGPSPYLSNIDLFLD	GKYITSVQ	GDGLIVSTPTGS GDGLIVSTPTGS	FAYAV.	AAGASMIHSSVPAIMVTPI AAGASMIHPSVPAIMVTPI	C PHSLS
	dwi_Dwi1_GK15608_GK15608_gene_	VLNEVVI	DRGPSPYLSNIDLFLD	GKYITSVQ	GDGLIVSTPTGS	FAYAV	AAGASMIHPSVPAIMVTPI	CHESLS
	aag_AaeL_AAEL000278_poly_p_/at	VLNEVVI	DRGLSSYLSNIDLFLD	GKHITSVQ	GDGLIVSTPTGS	TAYSA.	AGASMIHPSVPAILVTPI	CHESLS
	cqu_CpipJ_CPIJ018515_poly_p_/A	VLNEVVI	DRGMSSYLTNIDLFLD	GKHITSVQ	GDGLIVSTPTGS	FAYSA	AGASMIHPSVPAILVSPI	CPHSLS
NADK homologs,	isc_IscW_ISCW019567_sugar_kina	VLNEVVV	DRGPSPYLSNIDLYLD	GKLITTVQ	GDGLIVSTPTGS	FAYAV	AGASMIHPSVPAIMVTPI	CHESLS
possibly NADKs	bfo_BRAFLDRAFT_124524_hypothet	VLNEVVI	DRGPSPYLCHLDLYLE	GRHVTSVQ	GDGLIVSTPTGS	FAYAV	AAGASMVHPNVPAIMVTPI	CHISLS
	dre_550242_zgc_110083_EC_2.7.	VLNEVVI	DRGPSSYLSNVDLFLD	GHLITTVQ	GDGVLVSTPTGS	TAYAV	AGASMIHPNVPAIMITPI	CHESLS
	nve_NEMVE_vlg93522_hypothetica	VLNEVVI	DRGQSPYLSNLEVYCN	DYHITSVO	GDGLIISTPTGS	FAYAV	AGASMVHPTVPAILITPIC	CHSLS
	ppp_PHYPADRAFT_129555_hypothet	VLNEVAL	HRGMSSNLSNLECYCD	GNFVTSVQ	GDGLILSTPSGS	TAYSI	AGGSMVHPQVPGILFTPI	CHESLS
	sbi_SORBI_03g046360 hypothetic	VLNEIAI VLNEVTI	DRGISSYLTNLEVYCD	SSFVTCVO	GDGLILSTPSGS	TAYSI	AAGGSMVHPQVPGILFTPIC	CHESLS
	zma_100281602_NAD_kinase_1K0	VLNEVT	DRGISSYLTNLEVYCD	SSFVTCVQ	GDGLIISTTSGS	TAYSI	AAGGSMVHPQVPGILFTPI	CHSLS
	zma_100285591_NAD_kinase_1K0 osa 4325416 Os01g0957000 K008	VLNEVTI VLNEVTI	DRGISSYLTNLEVYCD	SSFVTCVQ	SUGLIISTTSGS	TAYSI.	AAGGSMVHPQVPGILFTPI	CHESLS
	pop_POPTR_172884_NADH_kinase/N	VLNEVT	DRGISSFLANLECYCD	NSFVTRVQ	GDGLILSTTSGS	FAYSL	GAGGSMVHPQVPGILFTPI	CHSLS
	pop_POPTR_203132_NADH_kinase/N rcu_RCOM_1578630_poly_p_/ATP_N	VLNEVTI	DRGISSFLTNLECYCD	NSFVTCVQ	GDGLILSTTSGS GDGLILSTTSGS	TAYSI.	AAGGSMVHPQVPGILFTPI	CHESLS
	vvi_100243315_hypothetical_pro	VLNEVT	DRGISSFLTNLECYSD	GSFVTCVQ	GDGLILSTTSGS	FAYSI	AAGGSMVHPQVPGILFTPI	CHSLS
	ath_AT3G21070_NADK1NADK1NA ppp_PHYPADRAFT_56276_hypotheti	VLNEVTI	DRGISSYLTNLECYCD	NSFVTCVQ GHEVTIVO	GDGLILSTTSGS	FAYSI.	AAGGSMVHPQVPGILFTPIC	CHESLS
	vcn_VOLCADRAFT_59341_hypotheti	VLNEVVI	DRGISSFLTNLECYCD	GTFVTHVQ	GDGLIVATPTGS	FAYNI	AAGGSMVHPQVPGILFTPI	CHISLS
	pif_PITG_02750_NAD_kinase_put	ALNEIVI	DRGPSGALVELNCYCD	GLEITKIA	ADGIIIATPTG	FAYSI	SAGGSMAHPSVPSMLFTPI	CHILS
	kla_KLLAOF16885g_hypothetical_	VLNELTI	DRGPSPFISMLELYGD	GSLLTVAQ	ADGLIIATSTGS	FAYSL	SAGGSLVYPSVNAIAVTPI	CHILS
	1th_KLTH0C03322g_KLTH0C03322p_	VLNELT]	DRGPSAFISMLEVFGD	NSLLTVAQ	ADGLIIATPTGS	FAYSI	SAGGSLVYPSVNAIAVTPI	CHILS
	cgr_CAGL0I01386g_hypothetical_	ILNEVT	DRGPSPFISMLELYGD	GNLMTVAQ	ADGLIIATPTGE	STATSI STAYSI	SAGGSLIYPTVNAIAVTPI	CHILS
Utr1	sce_YJR049C_UTR1_ATP-NADH_kin	ILNEVT]	DRGPSPFLSMLELYGD	GSLMTVAQ	ADGLIAATPTGS	FAYSI	SAGGSLVCPTVNAIALTPI	C PHALS
	dha_DEHA2C13464g_DEHA2C13464p_	VLNELV	DRGPSPYVTQLELYGD	GSLLTVAQ	ADGLIVATPTGE	FAYSL	SAGGSLINPGVNAIAVTPI	CHILS
	pic_PICST_87580_UTR1NAD_kina	VLNELV1	DRGPSPYVTQLELYGD	GSLLTIAQ	ADGLIIATPTGS	FAYSI	SAGGSLVHPGVSAISVTPI	CHTLS
	cal_Ca019.7393_UTR1NAD_kinas	VLNELV	DRGPSPIVTHLELIGD	GSLLTVAQ	ADGLIIATPTGE	FAYSL	SAGGSLVHPGVSAISVTPI	CHILS
	lel_LELG_02732_hypothetical_pr	VLNELVV	DRGPSPFVTQLELYGD	GSLLTIPQ	ADGLIIATPTGS	FAYSI	SAGGSLVHPGVSAISVTPI	CHTLS
	ppa PAS chr4 0912 hypothetical_pr	ALNELTY	DRGPSPYVTQLELYGD DRGPSPWVSMLELYGD	GSLLTVAO	ADGLIIATPTGS	STAYSI STAYSI	SAGGSLVHPGVSAISVTPIC	CHTLS
	aor_A0090701000420_predicted_s	VLNELV1	DRGPSPYVSNLELYAD	NDFLTVVQ	ADGCILSTPTGS	FAYSI	SAGGSLIHPSIPGILLTPI	CPHTLS
	ang_An03g05090_hypothetical_pr pcs Pc20g05800 Pc20g05800 A	VLNELVI	DRGPSPYVSNLELYAD DRGPSPYVSNLELYAD	DELLTVVQ	ADGCIFSTPTGS ADGCIFSTPTGS	SFAYSI SFAYSI	SAGGSLIHPSIPGILLTPIC SAGGSLMHPSIPGILLTPIC	CENTLS
	mbr MONBRDRAFT 12055 hypotheti	LLNEVTI	DRGPSPYLTKLEVYVD	GEPVTTIQ	GDGLIVATPTGS	FAYSA	AGGSMVHPAVACILLTPV	CHNVT
Pos5	sce_YPL188W_POS5Mitochon cqr CAGL0G02893g hypothetical	AMNDIFI AMNDIFI	HRGNSPHLTNLDIFID HRGNSPHLTNLDIYID	GEFLTRTT GEYLTRTT	ADGVALATPTGS ADGVTLSTPTGS	FAYSI FAYSI	SAGGSIVSPLVPAILMTPIC SAGGSIVSPLVPSILLTPIC	C PRSLS
	lth_KLTH0H04818g_KLTH0H04818p_	AMNDIFI	HRGNSPHLTNLDIFID	GEYLTRIT	ADGVTLATPTGS	FAYSI	SAGGSIVSPLVPSILLTPI	CFRSLS
	<pre>KIA_KLLA0E19097g_hypothetical_ zro ZYR00G08206g hypothetical</pre>	AMNDIFI AMNDLFI	HRGNSPHLTNLDIYID HRGNSPHLANLDIFID	GEFMTRIT GDFLTRTS	ADGVTLSTPTGS	TAYSI TAYSI	SAGGSIVSPLVPSILLTPIC	CERSLS
	vpo_Kpol_1002p66_hypothetical_	AMNDIFI	HRGSAPHLAYLDVFID	GKYLTRIT	ADGVALSTPTGS	TAYSI	SAGGSIVSPLVPCILLTPI	CFRSLS
	pic_PICST_53028_POS5_protein_ dha DEHA2F02442g DEHA2F02442p	AMNDISI AMNDISI	HRGSQPNLTSLDIYID HRGSQPNLISLDIYID	NEFLTTT NEFLTTT	ADGIVFSTPTGS	TAYSI TAYSI	SAGGSITHPLVPCILLTPIC SAGGSITHPLVPCIILTPIC	CPRSLS CPRSLS
	clu_CLUG_01758_hypothetical_pr	AMNDISI	HRGSQPNLISLDIVID	SEFLTTT	ADGIILASPTGS	FAYSI	SAGGSITHPLVPCILMTPV	CFRSLS
	ppa_PAS_chr1-4_0124_Mitochondr cal Ca019.14092 POS5 involved	AMNDIVI	HRGSIPTLTTLDIFID HRGSSPNLTSLDIYID	GEFLTRIT NEFFTTTF	ADGISFSTPTGS ADGVIFATPTGS	TAYSI TAYSI	SAGGSIVHPLVKCILLTPI SSGGSITHPSVPCVLLTPI	C PRSLS
	cal_Ca019.6800_POS5_involved_	AMNDVTI	HRGSSPNLTSLDIVID	NEFFTTTF	ADGVIFATPTGS	TAYSI	SGGSITHPSVPCVLLTPI	CPRSLS
	cdu_CD36_86940_NADH_kinase_mi ctp_CTRG_05636_hypothetical_pr	AMNDVT	HRGSSPNLTSLDIVID HRASLPNLTSLDIVID	NEFFTTTF NEFFTTTF	ADGVIFATPTGS ADGVILATPTGS	TAYSI	SSGGSITHPSVPCVLLTPIC SAGGSITHPAVPCTLLTPIC	CERSLS
	lel_LELG_03024_protein_POS5_A	AMNDITI	HRASSPNLTAVDIVID	GEFFTTTY	SDGLIFSTPTGS	TAYSI	SAGGSITHPAVPCILLTPI	CERSLS
Fundal Pos5	pgu_PGUG_02264_hypothetical_pr	AMNDISI	HRGGQPNLTSLDVYLN	DEFFTTTT	GDGILCSSPTGS	TAYSI	SAGGSIVHPSVPCILLTPI	CPRSLL
i ungai Poso	yli_YALIOE17963g_YALIOE17963p_	AMNDINI	HRGAEPHLTKLDIHVD	GEFITRAI	ADGVAVAIPIGE	STAYSI:	SSGGSIVBPQVPSILLTPI	CERSLS
homologs,	afv_AFLA_048340_mitochondrial_	VLNEVLI	HRGKEPHLAVVDVYVG	GRFLTEAV	ADGIIISTPTGS	TAYSI	SEGGSIVHPLVPSVLLTPI	CARSLS
possibly NADHKs	ang_An17g02020_hypothetical pr	VEVLI	HRGKEPHLAVLDVYVG	GRFLTEAV	ADGIIISTPTGS	TAYSI	SEGGSIVHPLVPSVLLTPIC	CARSLS
······································	act_ACLA_009150_poly_p_/ATP_NA	VMNEVLI	HRGKEPHLAVVDVYVG	GRFLTEAV	ADGMIISTPTGS	FAYSI	SGGSIVHPLVPAVLLTPI	CARSLS
	afm_AFUA_5G05890_mitochondrial	VMNEVLI	HRGKEPHLAVVDVYVG	GRFLTEAV	ADGMIISTPTGS	FAYSI FAYSI	SSGGSIVHPLVPAVLLTPIC	CARSLS
	ani_AN8837.2_hypothetical_prot	VMNEILI	HRGKEPHLAILDVYVG	GRFLTEAV	ADGIIISTPTGS	TAYSI	SGGSIVHPLVPAVLLTPI	CARSLS
	pcs_rc20g00980_Pc20g00980_A_ cpw CPC735 027640 ATP-NAD kina	VMNELLI AMNEVVI	HRGKEPHLAVVDVFVG HRGKQPHLAIVEVYVG	GRFLTEAV GRFLTEAV	ADGIIISTPTGS ADGMIISTPTGS	TAYSI TAYSI	SEGGSIVHPLVPSILLTPI SEGGSIIHPLVPSLLLTPI	CARSLS
	cim_CIMG_06228_hypothetical_pr	AMNEVVI	HRGKQPHLAIVEVYVG	GRFLTEAV	ADGMIISTPTGS	TAYSI	SSGGSIIHPLVPSLLLTPI	CERSLS
	ure_UREG_04158_hypothetical_pr ssl_SS1G_12647_hypothetical_pr	AMNEVVI	HRGKQPHLAIVEVYVG HRGKEAHLAITEVFVN	GRFLTEAV NOFLTEAU	ADGMIISTPTGS	TAYSI	SEGGSIIHPLVPSLLLTPIC	CARSLS
	pno_SNOG_16449_hypothetical_pr	ALNEVTI	HRGSSPHLKIIDVYIN	NRFLTEAV	ADGMIISSPTGS	FAYSI	SSGGSIVHPLVPSLLLTPI	CFRSLS
	tml_GSTUM_00006557001_hypothet	AMNEVNI	HRGKSPHLAVVEVFVS	GRFLTEAV	ADGMIIATPTGS	TAYSI	SEGGSIIHPSVSSLLLTPI	CPRSLS
	spo_SPAC323.01c_mitochondrial	AINEIS AMNEMHI	HRGLSPHMAVLKVFVN	DKFLTETT	ADGLLISTPTGS	TAYSI	SSGGPIVHPSINALLLTPIC	CINSLS
	cci_CC1G_01965_NADH_kinase_A_	VMNEIAI	HRGASPHLNTIDIFVD	GQHLTEAV	SDGLIVSTPTGS	TAYSI	SAGGPIVHPSLSALVLTPIC	CERSLS
	<pre>LDC_LACBIDRAFT_296215_hypothet scm SCHCODRAFT 57592 hypotheti</pre>	VMNEIAI VMNEVAI	HRGSSPHLNTIDIFVD	GOHLTEAV	SUGLIVSTPTGS SDGLIMSTPTGS	TAYSI TAYSI	SAGGPIVHPSLSALVLTPIC SAGGPIVHPSLSAIVLTPIC	CERSLS
	uma_UM04539.1_hypothetical_pro	LMNEVTI	HRGREPHMTKIDAYVD	GQHLTQAI	SDGLIIATPTGS	TAYSI	SAGGPIVHPSVQSLVLTPI	CERSLS
	cnb_CNBC2410_hypothetical_prot	VMNEVAI	HRGRNTHLTVVDTYFD	GOHLTEAV	ADGILLSTPTGS	TAYSI	SAGGPISHPETDAFLLTPI	AFRSLS
	-++ amiging an analy analy and		AODODA NUODEOBUTY	CDETIMOR	SCOT PTOTA ACS	D B B MC	COCEUM DM	T dobt o

Fig. S4.



Fig. S5.