Supporting Information

Conserved amino acid residues of the NuoD segment important for structure and function of *Escherichia coli* NDH-1 (complex I)

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Supplemental Figure 1

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NuoCDEco                 1 MTDLTAQEPAWQTRDHLDDPVIGELRNRFGPDAFTVQATRTGVPVVWIKREQLLEVGDFL
NuoCDRsp                 1 -----------MSLDQAIPEALQALRTRFGAAVRAEQATGEAFPVLWLDASVWEAAHRFL
NuoDRca                  1 ------------------------------------------------------------
Nqo4Pde                  1 ------------------------------------------------------------
Nqo4Tth                  1 ------------------------------------------------------------
NuoDMtu                  1 ------------------------------------------------------------
49kYli                   1 ------------------------------------------------------------
49KNcr                   1 ------------------------------------------------------------
49KXla                   1 ------------------------------------------------------------
49KBta                   1 ------------------------------------------------------------
49KHsa                   1 ------------------------------------------------------------
HyfGEco                  1 --------MNVNSSSNRGEAILAALKTQFPGAVLDEERQTPEQVTITVKINLLPDVVQYL
HycEEco                  1 -----------MSEEKLGQHYLAALNEAFPGVVLDHAWQTKDQLTVTVKVNYLPEVVEFL
FpoDMma                  1 ------------------------------------------------------------



NuoCDEco                61 -KKLPKPYVMLFDLHGMDERLRTHREGLPAADFSVFYHLISIDRNRDIMLKVALAENDLH
NuoCDRsp                50 REEIAAPFPLLADLWAIDESLRQHRTGQPASRITLCSHLVSLVRNADLRLKLATDG---R
NuoDRca                  1 ------------------------------------------------------------
Nqo4Pde                  1 ------------------------------------------------------------
Nqo4Tth                  1 ------------------------------------------------------------
NuoDMtu                  1 ------------------------------------------------------------
49kYli                   1 --------------------------------------MLRSAAARAVRAVRPR------
49KNcr                   1 ----------------------------------MATTLFRLAGRNAKRHCMRQSTTIAH
49KXla                   1 -----------------------------------------MAALRALGRLR--------
49KBta                   1 -----------------------------------------MAALRALCRLRGA------
49KHsa                   1 -----------------------------------------MAALRALCGFRGV------
HyfGEco                 53 YYQHDGWLPVLFG---------NDERTLNGHYAVYYALSMEGAEKCWIVVKALVDADSRE
HycEEco                 50 YYKQGGWLSVLFG---------NDERKLNGHYAVYYVLSMEKGTKCWITVRVEVDANKPE
FpoDMma                  1 ------------------------------------------------------------



NuoCDEco               120 VPTFTKLFPNANWYERETWDLFGITFDGHPNLRRIMMPQTWKGHPLRKDYPARATEFSPF
NuoCDRsp               107 APSIAGVYANADWYEREAHDMFGLDFG--RETRRILMPPTWEGHPLLKTHYARATEKPPF
NuoDRca                  1 ------------------------------------------------------------
Nqo4Pde                  1 ------------------------------------------------------------
Nqo4Tth                  1 ------------------------------------------------------------
NuoDMtu                  1 ------------------------------------------------------MTAIAD
49kYli                  17 --LSARYMA-----------------------TTALPQDPIPSGALGQKVPHVDESHQDL
49KNcr                  27 NLNSTRAFS-----------------------ASALRRYAEPS-YEGQGTRLVPTGDDFA
49KXla                  12 --APSGLLS-----------------------GRWLKLP-QSVRGK-QWQPDVEWTEQYA
49KBta                  14 --AAQVLRP-----------------------GAGVRLPIQPSRGARQWQPDVEWAEQYG
49KHsa                  14 --AAQVLRP-----------------------GAGVRLPIQPSRGVRQWQPDVEWAQQFG
HyfGEco                104 FPSVTPRVPAAVWGEREIR-------------DMYGLIPVGLPDQRRLVLPDDWPEDMHP
HycEEco                101 YPSVTPRVPAAVWGEREVR-------------DMYGLIPVGLPDERRLVLPDDWPDELYP
FpoDMma                  1 ------------------------------------------------------------
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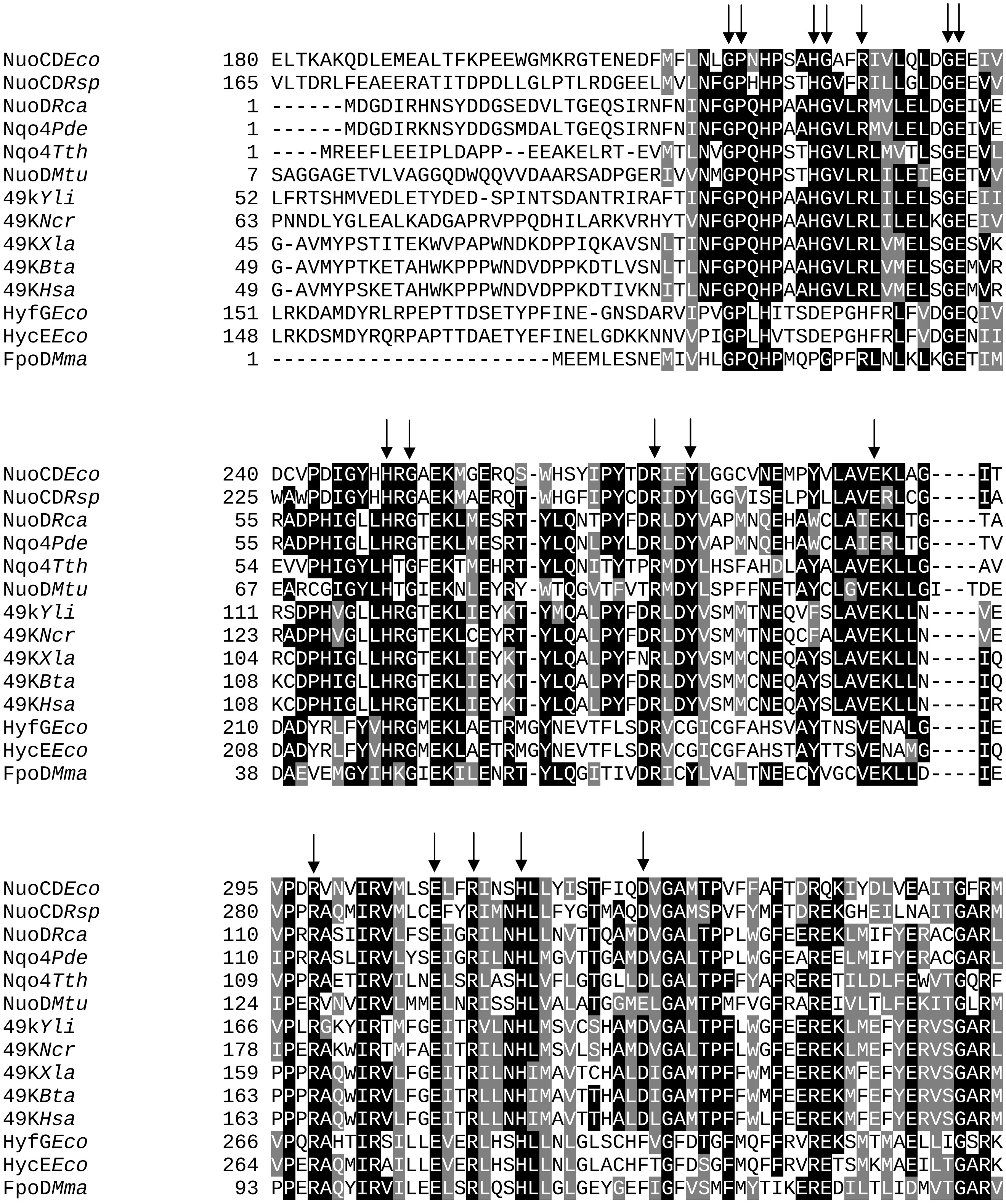

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NuoCDEco   180 ELTKAKQDLEMEALTFKPEEWGMKRGTENEDFMFLNLGPNHPSAHGAFRIVLQLDGEEIV
NuoCDRsp   165 VLTDRLFEAEERATITDPDLLGLPTLRDGEELMVLNFGPHHPSTHGVFRILLGLDGEEVV
NuoDRca      1 ------MDGDIRHNSYDDGSEDVLTGEQSIRNFNINFGPQHPAAHGVLRMVLELDGEIVE
Nqo4Pde      1 ------MDGDIRKNSYDDGSMDALTGEQSIRNFNINFGPQHPAAHGVLRMVLELDGEIVE
Nqo4Tth      1 ----MREEFLEEIPLDAPP--EEAKELRT-EVMTLNVGPQHPSTHGVLRLMVTLSGEEVL
NuoDMtu      7 SAGGAGETVLVAGGQDWQQVVDAARSADPGERIVVNMGPQHPSTHGVLRLILEIEGETVV
49kYli      52 LFRTSHMVEDLETYDED-SPINTSDANTRIRAFTINFGPQHPAAHGVLRLILELSGEEII
49KNcr      63 PNNDLYGLEALKADGAPRVPPQDHILARKVRHYTVNFGPQHPAAHGVLRLILELKGEEIV
49KXla      45 G-AVMYPSTITEKWVPAPWNDKDPPIQKAVSNLTINFGPQHPAAHGVLRLVMELSGESVK
49KBta      49 G-AVMYPTKETAHWKPPPWNDVDPPKDTLVSNLTLNFGPQHPAAHGVLRLVMELSGEMVR
49KHsa      49 G-AVMYPSKETAHWKPPPWNDVDPPKDTIVKNITLNFGPQHPAAHGVLRLVMELSGEMVR
HyfGEco    151 LRKDAMDYRLRPEPTTDSETYPFINE-GNSDARVIPVGPLHITSDEPGHFRLFVDGEQIV
HycEEco    148 LRKDSMDYRQRPAPTTDAETYEFINELGDKKNNVVPIGPLHVTSDEPGHFRLFVDGENII
FpoDMma      1 ----------------------MEEMLESNEMIVHLGPQHPMQPGPFRLNLKLKGETIM

NuoCDEco   240 DCVPDIGYHHRGAEKMGERQS-WHSYIPYTDRIEYLGGCVNEMPYVLAVEKLAG----IT
NuoCDRsp   225 WAWPDIGYHHRGAEKMAERQT-WHGFIPYCDRIDYLGGVISELPYLLAVERLCG----IA
NuoDRca     55 RADPHIGLLHRGTEKLMESRT-YLQNTPYFDRLDYVAPMNQEHAWCLAIEKLTG----TA
Nqo4Pde     55 RADPHIGLLHRGTEKLMESRT-YLQNLPYLDRLDYVAPMNQEHAWCLAIERLTG----TV
Nqo4Tth     54 EVVPHIGYLHTGFEKTMEHRT-YLQNITYTPRMDYLHSFAHDLAYALAVEKLLG----AV
NuoDMtu     67 EARCGIGYLHTGIEKNLEYRY-WTQGVTFVTRMDYLSPFFNETAYCLGVEKLLGI--TDE
49kYli     111 RSDPHVGLLHRGTEKLIEYKT-YMQALPYFDRLDYVSMMTNEQVFSLAVEKLLN----VE
49KNcr     123 RADPHVGLLHRGTEKLCEYRT-YLQALPYFDRLDYVSMMTNEQCFALAVEKLLN----VE
49KXla     104 RCDPHIGLLHRGTEKLIEYKT-YLQALPYFNRLDYVSMMCNEQAYSLAVEKLLN----IQ
49KBta     108 KCDPHIGLLHRGTEKLIEYKT-YLQALPYFDRLDYVSMMCNEQAYSLAVEKLLN----IQ
49KHsa     108 KCDPHIGLLHRGTEKLIEYKT-YLQALPYFDRLDYVSMMCNEQAYSLAVEKLLN----IR
HyfGEco    210 DADYRLFYVHRGMEKLAETRMGYNEVTFLSDRVCGICGFAHSVAYTNSVENALG----IE
HycEEco    208 DADYRLFYVHRGMEKLAETRMGYNEVTFLSDRVCGICGFAHSTAYTTSVENAMG----IQ
FpoDMma     38 DAEVEMGYIHKGIEKILENRT-YLQGITIVDRICYLVALTNEECYVGCVEKLLD----IE

NuoCDEco   295 VPDRVNVIRVMLSELFRINSHLLYISTFIQDVGAMTPVFFAFTDRQKIYDLVEAITGFRM
NuoCDRsp   280 VPPRAQMIRVMLCEFYRIMNHLLFYGTMAQDVGAMSPVFYMFTDREKGHEILNAITGARM
NuoDRca    110 VPRRASIIRVLFSEIGRILNHLLNVTTQAMDVGALTPPLWGFEEREKLMIFYERACGARL
Nqo4Pde    110 IPRRASLIRVLYSEIGRILNHLMGVTTGAMDVGALTPPLWGFEAREELMIFYERACGARL
Nqo4Tth    109 VPPRAETIRVILNELSRLASHLVFLGTGLLDLGALTPFFYAFREREТILDLFEWVTGQRF
NuoDMtu    124 IPERVNVIRVLMMELNRISSHLVALATGGMELGAMTPMFVGFRAREIVLTLFEKITGLRM
49kYli     166 VPLRGKYIRTMFGEITRVLNHLMSVCSHAMDVGALTPFLWGFEEREKLMEFYERVSGARL
49KNcr     178 IPERAKWIRTMFAEITRILNHLMSVLSHAMDVGALTPFLWGFEEREKLMEFYERVSGARL
49KXla     159 PPPRAQWIRVLFGEITRILNHIMAVTCHALDIGAMTPFFWMFEEREKMFEFYERVSGARM
49KBta     163 PPPRAQWIRVLFGEITRLLNHIMAVTTHALDIGAMTPFFWMFEEREKMFEFYERVSGARM
49KHsa     163 PPPRAQWIRVLFGEITRLLNHIMAVTTHALDLGAMTPFFWLFEEREKMFEFYERVSGARM
HyfGEco    266 VPQRAHTIRSILLEVERLHSHLLNLGLSCHFVGFDTGFMQFFRVREKSMTMAELLIGSRK
HycEEco    264 VPERAQMIRAILLEVERLHSHLLNLGLACHFTGFDSGFMQFFRVRETSMKMAEILTGARK
FpoDMma     93 PPERAQYIRVILEELSRLQSHLLGLGEYGEFIGFVSMFMYTIKEREDILTLIDMVTGARV
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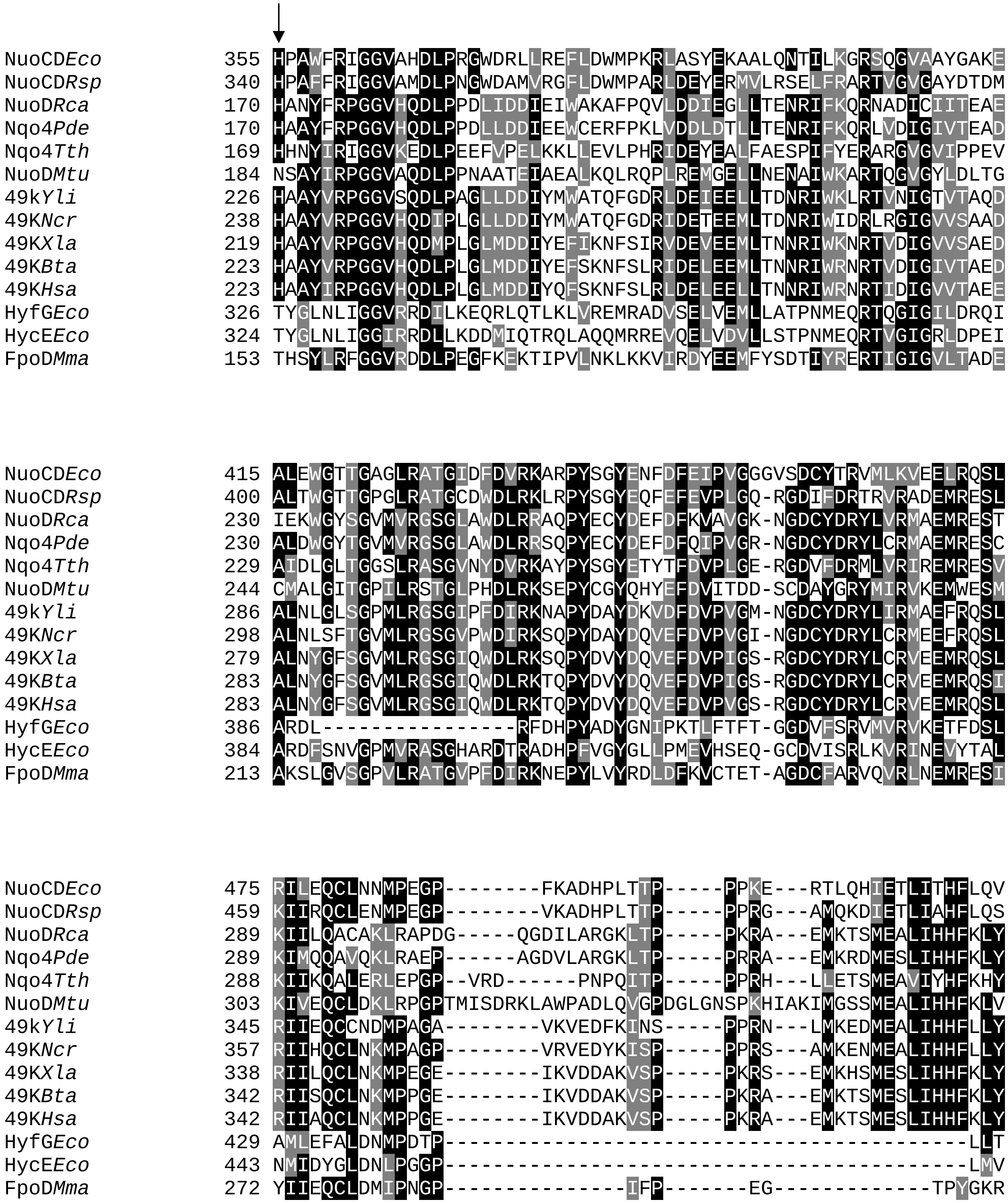

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NuoCDEco    355 HPAWFRIGGVAHDLPRGWDRLLREFLDWMPKRLASYEKAALQNTILKGRSQGVAAYGAKE
NuoCDRsp    340 HPAFFRIGGVAMDLPNGWDAMVRGFLDWMPARLDEYERMVLRSELFRARTVGVGAYDTDM
NuoDRca     170 HANYFRPGGVHQDLPPDLIDDIEIWAKAFPQVLDDIEGLLTENRIFKQRNADICIITEAE
Nqo4Pde     170 HAAYFRPGGVHQDLPPDLLDDIEEWCERFPKLVDDLDTLLTENRIFKQRLVDIGIVTEAD
Nqo4Tth     169 HHNYIRIGGVKEDLPEEFVPELKKLLEVLPHRIDEYEALFAESPIFYERARGVGVIPPEV
NuoDMtu     184 NSAYIRPGGVAQDLPPNAATEIAEALKQLRQPLREMGELLNENAIWKARTQGVGYLDLTG
49kYli      226 HAAYVRPGGVSQDLPAGLLDDIYMWATQFGDRLDEIEELLTDNRIWKLRTVNIGTVTAQD
49KNcr      238 HAAYVRPGGVHQDIPLGLLDDIYMWATQFGDRIDETEEMLTDNRIWIDRLRGIGVVSAAD
49KXla      219 HAAYVRPGGVHQDMPLGLMDDIYEFIKNFSIRVDEVEEMLTNNRIWKNRTVDIGVVSAED
49KBta      223 HAAYVRPGGVHQDLPLGLMDDIYEFSKNFSLRIDELEEMLTNNRIWRNRTVDIGIVTAED
49KHsa      223 HAAYIRPGGVHQDLPLGLMDDIYQFSKNFSLRLDELEELLTNNRIWRNRTIDIGVVTAEE
HyfGEco     326 TYGLNLIGGVRRDILKEQRLQTLKLVREMRADVSELVEMLLATPNMEQRTQGIGILDRQI
HycEEco     324 TYGLNLIGGIRRDLLKDDMIQTRQLAQQMRREVQELVDVLLSTPNMEQRTVGIGRLDPEI
FpoDMma     153 THSYLRFGGVRDDLPEGFKEKTIPVLNKLKKVIRDYEEMFYSDTIYRERTIGIGVLTADE


NuoCDEco    415 ALEWGTTGAGLRATGIDFDVRKARPYSGYENFDFEIPVGGGVSDCYTRVMLKVEELRQSL
NuoCDRsp    400 ALTWGTTGPGLRATGCDWDLRKLRPYSGYEQFEFEVPLGQ-RGDIFDRTRVRADEMRESL
NuoDRca     230 IEKWGYSGVMVRGSGLAWDLRRAQPYECYDEFDFKVAVGK-NGDCYDRYLVRMAEMREST
Nqo4Pde     230 ALDWGYTGVMVRGSGLAWDLRRSQPYECYDEFDFQIPVGR-NGDCYDRYLCRMAEMRESC
Nqo4Tth     229 AIDLGLTGGSLRASGVNYDVRKAYPYSGYETYTFDVPLGE-RGDVFDRMLVRIREMRESV
NuoDMtu     244 CMALGITGPILRSTGLPHDLRKSEPYCGYQHYEFDVITDD-SCDAYGRYMIRVKEMWESM
49kYli      286 ALNLGLSGPMLRGSGIPFDIRKNAPYDAYDKVDFDVPVGM-NGDCYDRYLIRMAEFRQSL
49KNcr      298 ALNLSFTGVMLRGSGVPWDIRKSQPYDAYDQVEFDVPVGI-NGDCYDRYLCRMEEFRQSL
49KXla      279 ALNYGFSGVMLRGSGIQWDLRKSQPYDVYDQVEFDVPIGS-RGDCYDRYLCRVEEMRQSL
49KBta      283 ALNYGFSGVMLRGSGIQWDLRKTQPYDVYDQVEFDVPIGS-RGDCYDRYLCRVEEMRQSI
49KHsa      283 ALNYGFSGVMLRGSGIQWDLRKTQPYDVYDQVEFDVPVGS-RGDCYDRYLCRVEEMRQSL
HyfGEco     386 ARDL----------------RFDHPYADYGNIPKTLFTFT-GGDVFSRVMVRVKETFDSL
HycEEco     384 ARDFSNVGPMVRASGHARDTRADHPFVGYGLLPMEVHSEQ-GCDVISRLKVRINEVYTAL
FpoDMma     213 AKSLGVSGPVLRATGVPFDIRKNEPYLVYRDLDFKVCTET-AGDCFARVQVRLNEMRESI


NuoCDEco    475 RILEQCLNNMPEGP--------FKADHPLTTP-----PPKE---RTLQHIETLITHFLQV
NuoCDRsp    459 KIIRQCLENMPEGP--------VKADHPLTTP-----PPRG---AMQKDIETLIAHFLQS
NuoDRca     289 KIILQACAKLRAPDG-----QGDILARGKLTP-----PKRA---EMKTSMEALIHHFKLY
Nqo4Pde     289 KIMQQAVQKLRAEP------AGDVLARGKLTP-----PRRA---EMKRDMESLIHHFKLY
Nqo4Tth     288 KIIKQALERLEPGP--VRD------PNPQITP-----PPRH---LLETSMEAVIYHFKHY
NuoDMtu     303 KIVEQCLDKLRPGPTMISDRKLAWPADLQVGPDGLGNSPKHIAKIMGSSMEALIHHFKLV
49kYli      345 RIIEQCCNDMPAGA--------VKVEDFKINS-----PPRN---LMKEDMEALIHHFLLY
49KNcr      357 RIIHQCLNKMPAGP--------VRVEDYKISP-----PPRS---AMKENMEALIHHFLLY
49KXla      338 RIILQCLNKMPEGE--------IKVDDAKVSP-----PKRS---EMKHSMESLIHHFKLY
49KBta      342 RIISQCLNKMPPGE--------IKVDDAKVSP-----PKRA---EMKTSMESLIHHFKLY
49KHsa      342 RIIAQCLNKMPPGE--------IKVDDAKVSP-----PKRA---EMKTSMESLIHHFKLY
HyfGEco     429 AMLEFALDNMPDTP--------------------------------------------LLT
HycEEco     443 NMIDYGLDNLPGGP--------------------------------------------LMV
FpoDMma     272 YIIEQCLDMIPNGP---------------IFP-------EG-------------TPYGKR
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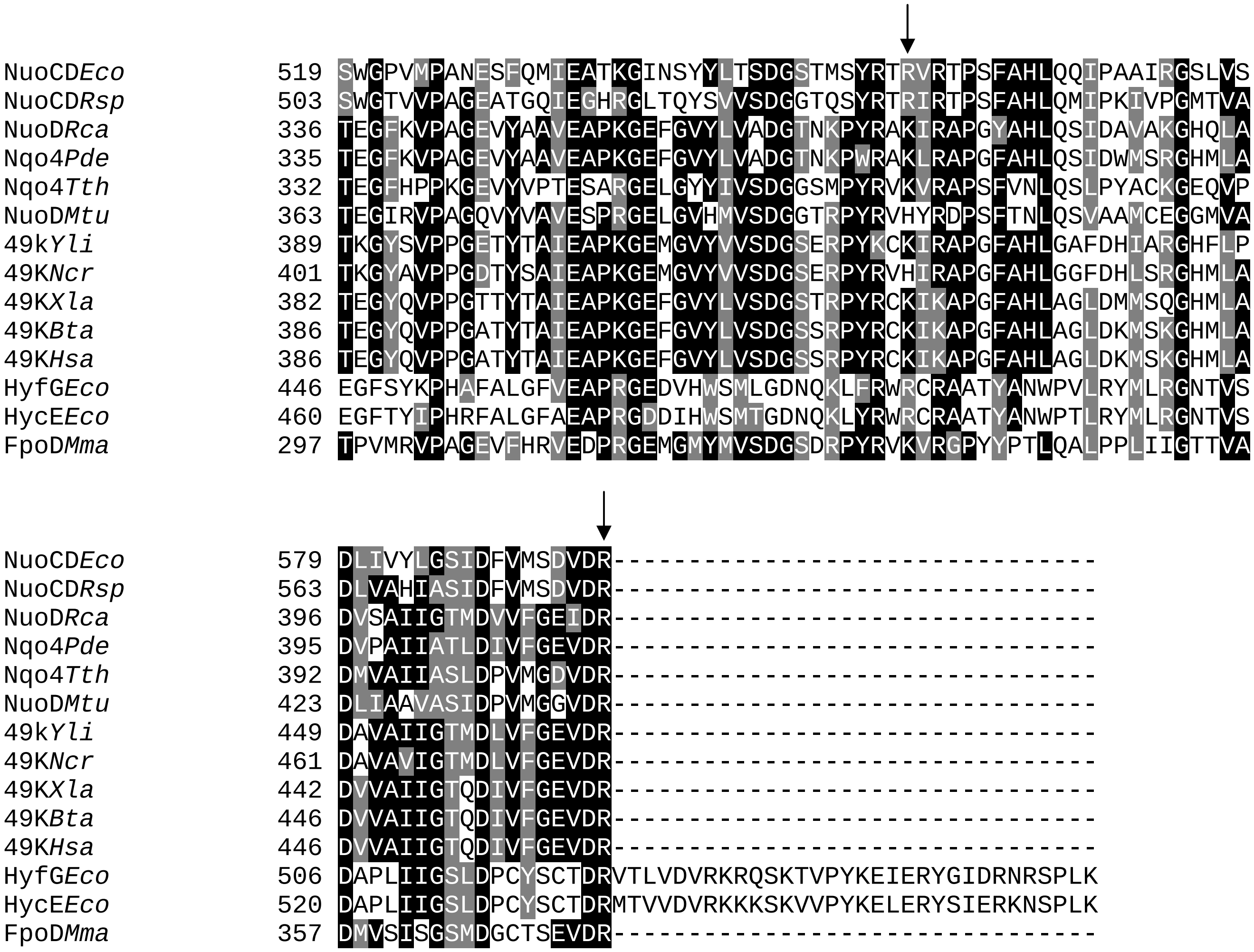
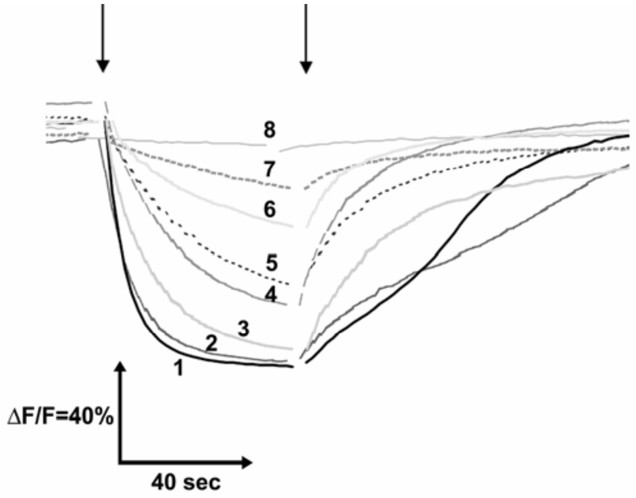


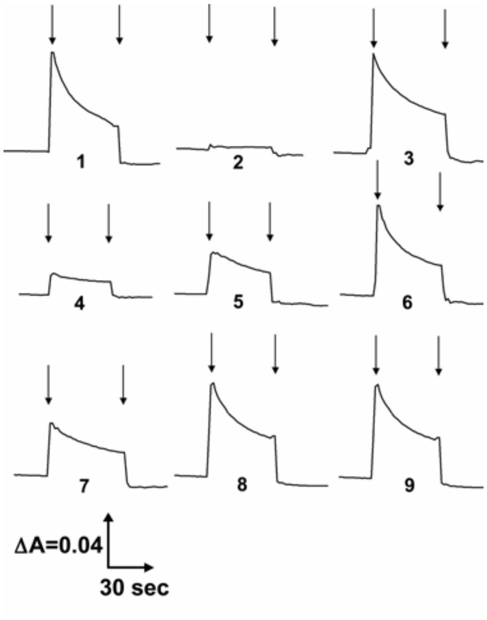
Supplemental Figure 1: Sequence alignment of the NuoD segment of *E. coli* NDH-1 with its homologues from other organisms. The alignment was done using ClustalW (61). The shading of the residues from the D segment was done by the default Box-shade program based on their similarity. Amino acids mutated in this study are marked by *arrows*. Sequence sources and their Swiss-Prot accession numbers are: NuoCD*Eco, E. coli* K-12 NuoCD subunit [P33599]; NuoCD*Rsp, R. spheroides* NuoCD subunit [B9KJI1]; NuoD*Rca, R. capsulatus* NuoD subunit [O07310]; Nqo4*Pde, P. denitrificans* Nqo4 subunit [A1B495]**;** Nqo4*Tth, T. thermophilus* HB-8 Nqo4 [Q56220]; NuoD*Mtu, Mycobacterium tuberculosis* NuoD subunit [A5U7G4]; 49K*Yli, Yarrowia lipolytica* NUCM subunit [Q9UUU1]; 49K*Ncr, Neurospora crassa* 49K subunit [P22142]; 49K*Xla, X. laevis* 49K subunit [Q32NR8]; 49K*Bta, Bos taurus* 49K subunit [P17694]; 49K*Hsa, Homo sapiens* 49K subunit [O75306]; HyfG*Eco, E. coli* HyfG subunit [P77329]; HycE*Eco, E. coli* HycE subunit [P16431]; FpoD*Mma, Methanosarcina mazei* F420H2 dehydrogenase FpoD subunit [Q9P9G0].

Supplemental Figure 2



Supplemental Figure 2. Generation of a pH gradient coupled to dNADH oxidation in *E. coli* NuoD mutants. Proton translocation was measured by the quenching of the fluorescence of ACMA at room temperature with an excitation wavelength of 410 nm and an emission wavelength of 480 nm. At the time indicated by arrows, 0.2 mM dNADH or 10 µM FCCP respectively, was added to the assay mixture containing 50 mM MOPS (pH7.3), 10 mM $MgCl_2$, 50 mM KCl, 2 µM ACMA, and *E. coli* membrane samples (50 µg of protein/ml). Representative traces from different group of mutants: 1, WT, KO-rev, H224R, G251A, R298K; 2, R228A, G235A, H315A, R228K, R298A, E308D; 3, D325A, G225A, R311K; 4, R556A, D325E, R270K; 5, H315R, Y273A, E236D ; 6, R270A, R311A; 7, E288Q, E288D, H355A ; 8, NuoD-KO, E308Q and G225V.

Supplemental Figure 3



Supplemental Figure 3. Detection of the membrane potential generated by dNADH oxidation in *E. coli* NuoD mutants. The membrane potential assay mixture contained 50 mM MOPS (pH 7.3), 10 mM $MgCl_2$, 50 mM KCl, 2 µM oxonol VI, and *E. coli* membrane samples (330 µg of protein/ml). The absorbance changes of oxonol VI were observed at 630-603 nm at 37 °C. The first arrow shows addition of 0.2 mM dNADH and the second arrow indicates addition of 2 µM FCCP to the assay mixture. Representative traces from different group of mutants: 1, WT, G251A and R298K; 2, NuoD-KO, G225V, E288Q, E288D, E308Q and H355A; 3, KO-rev; 4, E288D, H315R; 5, G225A, R311K, D325E; 6, R298A, R228K; 7, D325A, ; 8, H315A, E308D; 9, R270K.