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Supporting information for article:

Cryo-EM structure of *Neurospora crassa* respiratory complex IV

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N. crassa Cytochrome c oxidase subunit 1 (named Cox1), mitochondrially encoded
61.17 % identity with *S. cerevisiae* Cytochrome c oxidase subunit 1 (chain a in PDB 6HU9)

Figure S1 Sequence alignment of Cox1 from *N. crassa* and *S. cerevisiae* with Clustal Omega (1.2.4). Bold letters show transmembrane helices visible in the map. *N. crassa* has a longer C-terminus than *S. cerevisiae*.

N. crassa Cytochrome c oxidase subunit 2 (named Cox2), mitochondrially encoded
57.087 % identity with S. cerevisiae Cytochrome c oxidase subunit 2 (chain b in PDB 6HU9)

```

SP|P00411|COX2_NEUCR ---MGLLFNNLIMNFDAPSPWGIYFQDSATPQMEGLVELHDNIMYYLVVILFGVGWILL 56
SP|P00410|COX2_YEAST MLDLLRLQLTTF-IMNDVPTPYACYFQDSATPNQEGILEELHDNIMFYLLVILGLVSWMLY 59
      : * : . : *.*:*. *****: *:*****:***:*** *.*:*

SP|P00411|COX2_NEUCR SIIRNYISTKSPISHKYLNHGTLIELIWTITPAVILILIAFPFSKLLYLMDEVDPSMSV 116
SP|P00410|COX2_YEAST TIVMTY--SKNPIAYKYIKHGQTIEVIWTIFPAVILLIAFPFSFILLYLCDEVISPAMTI 117
      :*: . * :.*.***:***:*** **:**** *****:***** *** .*:**

SP|P00411|COX2_NEUCR LAEGHQWYWSYQYPDFLDSNDEFIEDSYIVPESDLEEGALRMLEVDRVILPELTHVRF 176
SP|P00410|COX2_YEAST KAIGYQWYWKEYSDFINDSGETVEFESYVIPDELLEGQLRLLDTSMVVPUVDTHIRF 177
      * *:****.*:***:*** * :***:***:***. *** *:***. *. ::* ***:***

SP|P00411|COX2_NEUCR IITAGDVIHSFAVPSLGVKCDAYPGRNLNQSVFVINREGVFYQGCSEICGILHSSMPIVIE 236
SP|P00410|COX2_YEAST VVTAADVIHDFAIPSLGKVDATPGRNLNQSVSALIQREGVFYGACSELCGTGHANMPIKIE 237
      :***.****.*:****:*** * ** *****:*****. :*:***** ***:*** *:.*** **

SP|P00411|COX2_NEUCR SVSLEKFLTGLEEQ 250
SP|P00410|COX2_YEAST AVSLPKFLEWLNEQ 251
      :*** *** ***:**

```

N. crassa Cytochrome c oxidase subunit 3 (named Cox3), mitochondrially encoded
53.532 % identity with S. cerevisiae Cytochrome c oxidase subunit 3 (chain c in PDB 6HU9)

```

SP|P00422|COX3_NEUCR MTNLIRSNFQDHFPFLVSPSPWPLNTSVCLLNLTGALSMHNFNNIHYLYYIALIGLVS 60
SP|P00420|COX3_YEAST MTHLERSRHQQHPFHVMVMPSPWPIVVSFALLSLALSTALTMHGYIGNMNMVYLALFVLLT 60
      ***:***..*:****:***:*****: . *...**.: : ***:***. . : :***: *:**

SP|P00422|COX3_NEUCR AMFLWFRDI ISEGTFLGDHTLAVQRGLNLGIILFIVSEALFFLAIFWAFFHSALTPTVEL 120
SP|P00420|COX3_YEAST SSILWFRDIVAEATYLGDHTMAVRKGINLGFLMFLSEVLIFAGLFWAYFHSAMSPDVTL 120
      : :*****:***.***:****:***:***:***:***:***.***: . :***:****:*** * * *

SP|P00422|COX3_NEUCR GAQWPPIGIEPVNPFELPLLNTVILLSSGATITYAHHALIKGEREGALYGSIATILLAI 180
SP|P00420|COX3_YEAST GACWPVGIEAVQPTELLPLLNTIILLSSGATVTYSHHALIAGNRNKALSGLLITFWLIVI 180
      *** ***:*** *:***:*****:*****:*****:***** *:***:*** *: *: * : *: * : *

SP|P00422|COX3_NEUCR FTGFQGVEYSVSSFTISDGAFGTCFFFSTGFHGIHVIGTIFLAVALWRIFAYHLTDNH 240
SP|P00420|COX3_YEAST FVTCQYIEYTNAAFTISDGVYGSVFYAGTGLHFLHMVMLAAMLGVNYYWRMRNYHLTAGHH 240
      *. * :***: :*****:***:***:***:***:***:***:***: ***: *** .***

SP|P00422|COX3_NEUCR VGFEGGILYWHFVVWLFYISVYYWGS 269
SP|P00420|COX3_YEAST VGYETIIYTHVLDFLYVVFYWWGV 269
      ***:***:***:***:***:***:***: .*:**

```

Figure S2 Sequence alignment of *N. crassa* and *S. cerevisiae* Cox2 and Cox3 with Clustal Omega (1.2.4). Bold letters show transmembrane helices visible in the map, grey letters show amino acids absent in the *S. cerevisiae* model (PDB 6HU9).

N. crassa Cytochrome c oxidase subunit 4 (named Cox4), nuclear-encoded

41.176 % identity with *S. cerevisiae* Cytochrome c oxidase subunit 4 (chain d in PDB 6HU9)

```

SP|P06809|COX4_NEUCR PGTVPDTDLEQATGLERLEILGKMEGVDFDMKPILDASRRGTMENPISVRSAGDEQYAGCT 119
SP|P04037|COX4_YEAST EGTVPDTDLDQETGLARLELLGKLEGIDVFDTKPLDSSRKGTMKDPIIESYDDYRYVGCT 112
*****;* *** *;***;*;*** *;*** .***;*;*** ;** ;* .* ;* ;*;***
```

SP|P06809|COX4_NEUCR GFPADSHNVIWLTMTTRPVERCPECGNVYKMDYVGQDDHAAHDHGHDHGFEFPKTFAD 179
 SP|P04037|COX4_YEAST GSPAGSHTIMWLKPTV-NEVARCWECSVYKLNPVGVPNDHHH----- 155
 * * * * ; * * ; * - * * * * * * ; * * ; * * ; *

SP|P06809|COX4_NEUCR YVKPEYW 186
SP|P04037|COX4 YEAST -----

***N. crassa* Cytochrome c oxidase subunit 6 (named Cox6), nuclear-encoded**

51.656 % identity with *S. cerevisiae* Cytochrome c oxidase subunit 6 (chain f in PDB 6HU9)

SP|Q01359|COX6_NEUCR MASFFRTAVRGPSAGLFRAVARPQP---IAARVSLFSTSSRFRSEHHEETFEEFTARYEK 57
SP|P00427|COX6_YEAST --MLSRAIFRNPVINRTLARPGAYHATRLTKNTFIQSRSKYSDAHDEETFEEFTARYEK 58
..... * * * * *** * * .. * *****

SP|Q01359|COX6_NEUCR GQEYQYLAEKLPLREELGITLKDLYPEEAN 148
SP|P00427|COX6_YEAST DQKYAKYLDELKDVQRQELGVPLKEELFPSS- 148
.....***: *** ***: *:****: ***:***: ..

***N. crassa* Cytochrome c oxidase subunit (named Cox6b), nuclear-encoded**

53.488 % identity with *S. cerevisiae* Cytochrome c oxidase subunit 6B (chain i in PDB 6HU9)

```

TR|Q1K8U2|Q1K8U2_NEUCR  MSDDERVTKPKFVTVGDARFPNVNQTKHCKWQNQYVDYHKCILAKGEDFAPCRQFWLAYRS 60
SP|Q01519|COX12_YEAST  MADQEN---SPLHTVGFNDARFPQQNQTKHCKWQSVDYHKCVNMKGEDFAPCKVFWKTYNA 57
* * * *
* * * * *
* * * * *
* * * * *
* * * . *

```

TR|Q1K8U2|Q1K8U2_NEUCR LCPSGWYQRWDQEAGNFPVKLE-- 84
SP|Q01519|COX12_YEAST LCPLDWIEKWDQQREKGIFAGDINS
 *** . * ; ***; *** * * . .

Figure S3 Sequence alignments of soluble subunits Cox4, Cox6 and Cox6b from *N. crassa* and *S. cerevisiae* with Clustal Omega (1.2.4). Grey letters indicate residues that are absent in the *S. cerevisiae* model (PDB 6HU9).

***N. crassa* Cytochrome c oxidase polypeptide 5 (named Cox5a), nuclear-encoded
29.31 % identity with *S. cerevisiae* Cytochrome c oxidase polypeptide 5a (chain e in PDB 6HU9)**

```

SP|P06810|COX5_NEUCR MLRPTVSLVRNAVRAA--KPTMAVRAASTMPISNPTLANIEKRWEQMPMQEQAELWM 58
SP|P00424|COX5A_YEAST -----MLRNFTTRAGGLSRTSVRAQTHALNSAAVMDLQSRWENMPSTEQQDIVS 51
                                         ::**. .**. . :** *.* :** :: ::::****:*** ** ::

SP|P06810|COX5_NEUCR ALRDRMKGNWADLTQEKKAAYYIAFGPHGPRALPPP-GEQKKVILAYTVAGVFLSFVIFA 117
SP|P00424|COX5A_YEAST KLSERQKLPWAQLTEPEKQAVWYISYGEWPGRPPVLNKGDSSFIAKGVAAGLLFSGVGLFA 111
                                         * :* * ***:*** **:***:*** *** *:... : ..***:***. :**

SP|P06810|COX5_NEUCR TMRAFAKPPPATMTKEWQEATNEFLKAQKSDPLTGLTSEGYNGKGHVQSPSASA 171
SP|P00424|COX5A_YEAST VVRMAGGQDAKTMNKEWQLKSDEYLKSKNANPWGGYSQVQSK----- 153
                                         .:*. . ***.***** :*:***:***:*** * .. :
```

***N. crassa* Uncharacterized protein (named Cox7), nuclear-encoded
15.493 % identity with *S. cerevisiae* Cytochrome c oxidase subunit 7 (chain g in PDB 6HU9)**

```

TR|Q7S7U5|Q7S7U5_NEUCR MPGLVNAPNHPKEQRYYQQAFKNHTRLWKGPRSGIIMTTFNIAMWGTGASMYAMSRK 60
SP|P10174|COX7_YEAST -----MANKVIQLQKIFQS---STKPLWWRHPRSALYLYPFYAIFAVAVVTPLLYIPNA 51
                                         *: * : * :*. . . ** ***.: : * : .: .: .: . .

TR|Q7S7U5|Q7S7U5_NEUCR VLYGNTWFSED 71
SP|P10174|COX7_YEAST IRGIKAKKA-- 60
                                         : * : : :
```

***N. crassa* Cytochrome c oxidase chain VIIc (named Cox8), nuclear-encoded
16.471 % identity with *S. cerevisiae* Cytochrome c oxidase polypeptide VIII (chain h in PDB 6HU9)**

```

TR|Q1K528|Q1K528_NEUCR MFSRVALRAAPRQQPFSLVARRTFQTTRAQLSSPYHYPEGRSNSLPNPKTRFFFWFRY-- 58
SP|P04039|COX8_YEAST MLCQQMIRTTAKRS-----SNIMTRPIIMKRSVHFKDGVYENIPFKVKGRKTPYALSH 53
                                         *: : *: : :. .: * :. *: :* .:***: * * :
```

```

TR|Q1K528|Q1K528_NEUCR LMYCVVGFGSPVAIAVWQTYRPRS- 82
SP|P04039|COX8_YEAST FGFFAIGFAVPFVACYVQLKKSGAF 78
                                         : : .:***. *... . * : :
```

***N. crassa* Cytochrome c oxidase subunit 7A (named Cox7a), nuclear-encoded
32.812 % identity with *S. cerevisiae* Cytochrome c oxidase subunit 7A (chain j in PDB 6HU9)**

```

TR|Q7S5M7|Q7S5M7_NEUCR MAATAVRPITGMLRRGLILDIGIALGVGFVMANGYWYGYHMPRTNARDNYKKLEEERA 60
SP|P07255|COX9_YEAST ---MTIAPITGTIKRRVIMDIVLGFSLLGGVMASYWWGFHMDKINKREKFYAELAERKKQ 57
                                         : : **** :* :*:*** :.: .: * .:***: * : * : :* .:
```

```

TR|Q7S5M7|Q7S5M7_NEUCR RMGA 64
SP|P07255|COX9_YEAST EN-- 59
                                         .
```

Figure S4 Sequence alignments of *N. crassa* and *S. cerevisiae* subunits Cox5a, Cox7, Cox8 and Cox7a with Clustal Omega (1.2.4). Grey letters indicate residues that are absent in the *S. cerevisiae* model (PDB 6HU9), bold letters mark transmembrane regions. All proteins have one transmembrane helices.

***N. crassa* Encodes anonymous transcript-5 protein (named Cox6a), nuclear-encoded
33.813 % identity with *S. cerevisiae* Cytochrome c oxidase subunit 6A, named Cox13 (chain k in PDB 6HU9)**

TR|V5IRD7|V5IRD7_NEUCR MFAQRQMFFARLAANLRAPAVRQTVQRRFASTPANE SGKNAFVREREAVKQHAAE**TTELW** 60
 SP|P32799|COX13_YEAST **MFRQC**A KRY---ASSLPPN---ALKP--AFGPPDKVAAQKFKE SLMATEKHAKD**TSNMW** 51
 *** * : *.* :: * * : : . : * .. *.:** :*::*

TR|V5IRD7|V5IRD7_NEUCR **RKISILY**GIPPALALAGYNAYTLYNEHWE---HW SHLPPL EERTE YPYQNIR TRNYPWG D 117
 SP|P32799|COX13_YEAST **VKISVW**VALPAIALTA VNTYFVEKEHAEHREHLKHVPDSE WPRDYEFM NIRS KPF FWGD G 111
 : : **::.. *: * : :** * * .*: * * : * : ***: : ****

TR|V5IRD7|V5IRD7_NEUCR DKT LFWN ESVNYHN RDKV T 136
 SP|P32799|COX13_YEAST DKT LFWN P VNRHIEHDD- 129
 ***** * * ...

***S. cerevisiae* Uncharacterized protein YDR119W-A, named Cox26 (chain l in PDB 6HU9)
absent in *N. crassa* genome**

sp|Q2V2P9|YD19A_YEAST MFFSQVL RSSARA APIKRYTG RIGESW VITEGRRLIPEIF QWSA VLSVCLGWP GAVYFFSKARKA 66

Figure S5 Sequence alignment of *N. crassa* subunit Cox6a and *S. cerevisiae* subunit Cox13 with Clustal Omega (1.2.4), and sequence of *S. cerevisiae* subunit Cox26. Grey letters indicate residues that are absent in the *S. cerevisiae* model (PDB 6HU9), bold letters mark transmembrane regions. *S. cerevisiae* Cox6a has a homologue in *N. crassa* that is not present in the Cryo-EM map. *N. crassa* does not have a Cox26 homologue, and no density is visible in the Cryo-EM map.