Supplemental Data for Zickermann et al.

ALIGNMENTS

All alignments were done using the programme CLUSTAL W. The sequences of the two epitopes identified in this study are underlined and printed in bold. In alignments B and C the Ni ligating cysteines are shown in green.

A: Sequences of the 49 kDa subunit from *Yarrowia lipolytic*a, *Neurospora crass*a, *Bos taurus*, *Homo sapien*s, *Paracoccus denitrificans*, *Rhodobacter capsulatus*, *Thermus thermophilus* and *Escherichia coli*. For *E. coli* only the part of the NuoCD subunit (fusion of 30 kDa and 49 kDa subunit) corresponding to the 49 kDa subunit from other organisms was included in the alignment.

B: EchE subunit of the membrane bound hydrogenase from *Methanosarcina barkeri* aligned with the 49 kDa subunit from *Yarrowia lipolytica* complex I.

C: 49 kDa subunit of complex I from *Y. lipolytica* aligned with the EchE subunit of the membrane bound [NiFe] hydrogenase from *M. barkeri* and the large subunit of the soluble [NiFe] hydrogenase from *Desulfovibrio fructosovoran*s.

Alignment A

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49_kD_Y.lipolytica         ATTALPQDPIPSGALGQKVPHVDESHQDLLFRTSHMVEDLETYDEDSPIN 50
49_kD_N.crassa             ------AEPSYEGQGTRLVPTGDDFAPNNDLYGLEALKADGAPR--VPPQ 42
49_kD_B.taurus             --------------ARQWQPDVEWAEQYGGAVMYPTKETAHWKPPPWNDV 36
49_kD_H.sapiens            --------------VRQWQPDVEWAQQFGGAVMYPSKETAHWKPPPWNDV 36
49_kD_P.denitrificans      ----------------------------------MDGDIRKNSLDDGSMD 16
49_kD_Rh.capsulatus        ----------------------------------MDGDIRHNSYDDGSED 16
49_kD_Th.thermophilus      ---------------------------------MREEFLEEIPLDAPP 15
49_kD_part_E.coli          -------------------------------------MEALTFKPEEWG 12

49_kD_Y.lipolytica         TSDANTRIRAFTINFGPQHPAAHGVLRLILELSGEEIIRSDPHVGLLHRG 100
49_kD_N.crassa             DHILARKVRHYTVNFGPQHPAAHGVLRLILELKGEEIVRADPHVGLLHRG 92
49_kD_B.taurus             DPPKDTLVSNLTLNFGPQHPAAHGVLRLVMELSGEMVRKCDPHIGLLHRG 86
49_kD_H.sapiens            DPPKDTIVKNITLNFGPQHPAAHGVLRLVMELSGEMVRKCDPHIGLLHRG 86
49_kD_P.denitrificans      ALTGEQSIRNFNINFGPQHPAAHGLLRMVLELDGEIVERADPHIGLLHRG 66
49_kD_Rh.capsulatus        VLTGEQSIRNFNINFGPQHPAAHGVLRMVLELDGEIVERADPHIGLLHRG 66
49_kD_Th.thermophilus      EEAKELRTEVMTLNVGPQHPSTHGVLRLMVTLSGEEVLEVVPHIGYLHTG 65
49_kD_part_E.coli          MKRGTENEDFMFLNLGPNHPSAHGAFRIVLQLDGEEIVDCVPDIGYHHRG 62

49_kD_Y.lipolytica         TEKLIEYKTYMQALPYFDRLDYVSMMTNEQVFSLAVEKLLNVEVPLRGKY 150
49_kD_N.crassa             TEKLCEYRTYLQALPYFDRLDYVSMMTNEQCFALAVEKLLNVEIPERAKW 142
49_kD_B.taurus             TEKLIEYKTYLQALPYFDRLDYVSMMCNEQAYSLAVEKLLNIRPPPRAQW 136
49_kD_H.sapiens            TEKLIEYKTYLQALPYFDRLDYVSMMCNEQAYSLAVEKLLNIRPPPRAQW 136
49_kD_P.denitrificans      TEKLMESRTYLQNLPYLDRLDYVAPMNQEHAWCLAIERLTGTVIPRRASL 116
49_kD_Rh.capsulatus        TEKLMESRTYLQNTPYFDRLDYVAPMNQEHAWCLAIEKLTGTAVPRRASI 116
49_kD_Th.thermophilus      FEKTMEHRTYLQNITYTPRMDYLHSFAHDLAYALAVEKLLGAVVPPRAET 115
49_kD_part_E.coli          AEKMGERQSWHSYIPYTDRIEYLGGCVNEMPYVLAVEKLAGITVPDRVNV 112

49_kD_Y.lipolytica         IRTMFGEITRVLNHLMSVCSHAMDVGALTPFLWGFEEREKLMEFYERVSG 200
49_kD_N.crassa             IRTMFAEITRILNHLMSVLSHAMDVGALTPFLWGFEEREKLMEFYERVSG 192
49_kD_B.taurus             IRVLFGEITRLLNHIMAVTTHALDIGAMTPFFWMFEEREKMFEFYERVSG 186
49_kD_H.sapiens            IRVLFGEITRLLNHIMAVTTHALDLGAMTPFFWLFEEREKMFEFYERVSG 186
49_kD_P.denitrificans      IRVLYSEIGRILNHLMGVTTGAMDVGALTPPLWGFEAREELMIFYERACG 166
49_kD_Rh.capsulatus        IRVLFSEIGRILNHLLNVTTQAMDVGALTPPLWGFEEREKLMIFYERACG 166
49_kD_Th.thermophilus      IRVILNELSRLASHLVFLGTGLLDLGALTPFFYAFRERETILDLFEWVTG 165
49_kD_part_E.coli          IRVMLSELFRINSHLLYISTFIQDVGAMTPVFFAFTDRQKIYDLVEAITG 162
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49_kD_Y.lipolytica         ARLHAAYVRPGGVSQDLPAGLLDDIYMWATQFGDRLDEIEELLTDNRIWK 250
49_kD_N.crassa            ARLHAAYVRPGGVHQDIPLGLLDDIYMWATQFGDRIDETEEMLTDNRIWI 242
49_kD_B.taurus            ARMHAAYVRPGGVHQDLPLGLMDDIYEFSKNFSLRIDELEEMLTNNRIWR 236
49_kD_H.sapiens           ARMHAAYIRPGGVHQDLPLGLMDDIYQFSKNFSLRLDELEELLTNNRIWR 236
49_kD_P.denitrificans     ARLHAAYFRPGGVHQDLPPDLLDDIEEWCERFPKLVDDLDTLLTENRIFK 216
49_kD_Rh.capsulatus       ARLHANYFRPGGVHQDLPPDLIDDIEIWAKAFPQVLDDIEGLLTENRIFK 216
49_kD_Th.thermophilus     QRFHHNYIRIGGVKEDLPEEFVPELKKLLEVLPHRIDEYEALFAESPIFY 215
49_kD_part_E.coli         FRMHPAWFRIGGVAHDLPRGWDRLLREFLDWMPKRLASYEKAALQNTILK 212

49_kD_Y.lipolytica         LRTVNIGTVTAQDALNLGLSGPMLRGSGIPFDIRKNAPYDAYDKVDFDVP 300
49_kD_N.crassa            DRLRGIGVVSAADALNLSFTGVMLRGSGVPWDIRKSQPYDAYDQVEFDVP 292
49_kD_B.taurus            NRTVDIGIVTAEDALNYGFSGVMLRGSGIQWDLRKTQPYDVYDQVEFDVP 286
49_kD_H.sapiens           NRTIDIGVVTAEEALNYGFSGVMLRGSGIQWDLRKTQPYDVYDQVEFDVP 286
49_kD_P.denitrificans     QRLVDIGIVTEADALDWGYTGVMVRGSGLAWDLRRSQPYECYDEFDFQIP 266
49_kD_Rh.capsulatus       QRNADICIITEAEIEKWGYSGVMVRGSGLAWDLRRAQPYECYDEFDFKVA 266
49_kD_Th.thermophilus     ERARGVGVIPPEVAIDLGLTGGSLRASGVNYDVRKAYPYSGYETYTFDVP 265
49_kD_part_E.coli         GRSQGVAAYGAKEALEWGTTGAGLRATGIDFDVRKARPYSGYENFDFEIP 262

49_kD_Y.lipolytica         VGM-NGDCYDRYLIRMAEFRQSLRIIEQCCNDMPAGA---VKVEDFKINS 346
49_kD_N.crassa            VGI-NGDCYDRYLCRMEEFRQSLRIIHQCLNKMPAGP---VRVEDYKISP 338
49_kD_B.taurus            IGS-RGDCYDRYLCRVEEMRQSIRIISQCLNKMPPGE---IKVDDAKVSP 332
49_kD_H.sapiens           VGS-RGDCYDRYLCRVEEMRQSLRIIAQCLNKMPPGE---IKVDDAKVSP 332
49_kD_P.denitrificans     VGR-NGDCYDRYLCRMAEMRESCKIMQQAVQKLRAEP-AGDVLARGKLTP 314
49_kD_Rh.capsulatus       VGK-NGDCYDRYLVRMAEMRESTKIILQACAKLRAPDGQGDILARGKLTP 315
49_kD_Th.thermophilus     LGE-RGDVFDRMLVRIREMRESVKIIKQALERLEPGP---VRDPNPQITP 311
49_kD_part_E.coli         VGGGVSDCYTRVMLKVEELRQSLRILEQCLNNMPEGP---FKADHPLTTP 309

49_kD_Y.lipolytica         PPRNLMKEDMEALIHHFLLYTKGYSVPPGETYTAIEAPKGEMGVYVVSDG 396
49_kD_N.crassa            PPRSAMKENMEALIHHFLLYTKGYAVPPGDTYSAIEGPKGEMGVYVVSDG 388
49_kD_B.taurus            PKRAEMKTSMESLIHHFKLYTEGYQVPPGATYTAIEAPKGEFGVYLVSDG 382
49_kD_H.sapiens           PKRAEMKTSMESLIHHFKLYTEGYQVPPGATYTAIEAPKGEFGVYLVSDG 382
49_kD_P.denitrificans     PRRAEMKRDMESLIHHFKLYTEGFKVPAGEVYAAVEGPKGEFGVYLVADG 364
49_kD_Rh.capsulatus       PKRAEMKTSMEALIHHFKLYTEGFKVPAGEVYAAVEAPKGEFGVYLVADG 365
49_kD_Th.thermophilus     PPRHLLETSMEAVIYHFKHYTEGFHPPKGEVYVPTESARGELGYYIVSDG 361
49_kD_part_E.coli         PPKERTLQHIETLITHFLQVSWGPVMPANESFQMIEATKGINSYYLTSDG 359

49_kD_Y.lipolytica         SERPYKCKIRAPGFAHLGAFDHIARGHFLPDAVAIIGTMDLVFGEVDR 444
49_kD_N.crassa            SERPYRVHIRAPGFAHLGGFDHLSRGHMLADAVAVIGTMDLVFGEVDR 436
49_kD_B.taurus            SSRPYRCKIKAPGFAHLAGLDKMSKGHMLADVVAIIGTQDIVFGEVDR 430
49_kD_H.sapiens           SSRPYRCKIKAPGFAHLAGLDKMSKGHMLADVVAIIGTQDIVFGEVDR 430
49_kD_P.denitrificans     TNKPWRAKLRAPGFAHLQSIDWMSRGHMLADVPAIIATLDIVFGEVDR 412
49_kD_Rh.capsulatus       TNKPYRAKIRAPGYAHLQSIDAVAKGHQLADVSAIIGTMDVVFGEIDR 413
49_kD_Th.thermophilus     GSMPYRVKVRAPSFVNLQSLPYACKGEQVPDMVAIIASLDPVMGDVDR 409
49_kD_part_E.coli         STMSYRTRVRTPSFAHLQQIPAAIRGSLVSDLIVYLGSIDFVMSDVDR 407
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Alignment B

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M.barkeri_EchE         ------------------------------------------------------------MT 2
Y.lipolytica_49kD      ATTALPQDPIPSGALGQKVPHVDESHQDLLFRTSHMVEDLETYDEDSPINTSDANTRIRA 60

M.barkeri_EchE         TVIPFGPQHPVLPEPVSLKLEIDDNVVVGVLPSLGYVHRGLETFINTKDFNQTTYVCERI 62
Y.lipolytica_49kD      FTINFGPQHPAAHGVLRLILELSGEEIIRSDPHVGLLHRGTEKLIEYKTYMQALPYFDRL 120
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M.barkeri_EchE          CGICSALHGITYTRTVEKLFDTEIPERAQYIRVIVGELNRLHSHLLWLGLFADGFGFESL 122
Y.lipolytica_49kD       DYVSMMTNEQVFSLAVEKLLNVEVPLRGKYIRTMFGEITRVLNHLMSVCSHAMDVGALTP 180

M.barkeri_EchE          FYECWKYREEVLDVAERICGNRVIHSISKVGGVTRDLTKEHIDMLLKMCDSLETEIKNIE 182
Y.lipolytica_49kD       FLWGFEEREKLMEFYERVSGARLHAAYVRPGGVSQDLPAGLLDDIYMWATQFGDRLDEIE 240

M.barkeri_EchE          KVFVNNYTVKQRLVGLATLSKQVAYEVGTAGPTLRGSGNAIDVRETPDWDIYKDLGFKTA 242
Y.lipolytica_49kD       ELLTDNRIWKLRTVNIGTVTAQDALNLGLSGPMLRGSGIPFDIRKNAPYDAYDKVDFDVP 300

M.barkeri_EchE          VEKDGDCYARTKVRITELLNSLTIIRNAISKMPEGEIEVR------------------- 282
Y.lipolytica_49kD       VGMNGDCYDRYLIRMAEFRQSLRIIEQCCNDMPAGAVKVEDFKINSPPRNLMKEDMEALI 360

M.barkeri_EchE          --------VKGFPTGEAIMRTEQPRGEVIYYVKGNGTKKLERLKVRTPTFANIPSLLLML 334
Y.lipolytica_49kD       HHFLLYTKGYSVPPGETYTAIEAPKGEMGVYVVSDGSERPYKCKIRAPGFAHLGAFDHIA 420

M.barkeri_EchE          PGVKLADVPIVVLTIDPCVSCTER 358
Y.lipolytica_49kD       RGHFLPDAVAIIGTMDLVFGEVDR 444
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Alignment C

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Y.lipolytica_49kD       ATTALPQDPIPSGALGQKVPHVDESHQDLLFRTSHMVEDLETYDEDSPINTSDANTRIRA 60
M.barkeri_EchE          ------------------------------------------------------MT 2
D.fructosovorans_LS     -------------------------------------------------MAESKPTPQS 10

Y.lipolytica_49kD       FTINFGPQHPAAHGVLRLILELSGEE-IIRSDPHVGLLHRGTEKLIEYKTYM-QALPYFD 118
M.barkeri_EchE          TVIPFGPQHPVLPEPVSLKLEIDDNV-VVGVLPSLGYVHRGLETFINTKDFN-QTTYVCE 60
D.fructosovorans_LS     TFTGPIVVDPITRIEGHLRIMVEVENGKVKDAWSSSQLFRGLEIILKGRDPR-DAQHFTQ 69
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Y.lipolytica_49kD        RLDYVSMMTNEQVFSLAVEKLLNVEVPLRGKYIRTMFGEITRVLNHLMSVC--------- 169
M.barkeri_EchE           RICGICSALHGITYTRTVEKLFDTEIPERAQYIRVIVGELNRLHSHLLWLG--------- 111
D.fructosovorans_LS      RACGVCTYVHALASSRCVDDAVKVSIPANARMMRNLVMASQYLHDHLVHFYHLHALDWVD 129


Y.lipolytica_49kD        ----------------------------------------------------SHAMDVG--- 176
M.barkeri_EchE           ----------------------------------------------------LFADGFG--- 118
D.fructosovorans_LS      VTAALKADPNKAAKLAASIDTARTGNSEKALKAVQDKLKAFVESGQLGIFTNAYFLGGHK 189


Y.lipolytica_49kD        -----------ALTPFLWGFEEREKLMEFYERVSGARLHAAYVRPGGVS--QDLPAGLLD 223
M.barkeri_EchE           -----------FESLFYECWKYREEVLDVAERICGNRVIHSISKVGGVT--RDLTKEHID 165
D.fructosovorans_LS      AYYLPPEVNLIATAHYLEALHMQVKAASAMAILGGKNPHTQFTVVGGCSNYQGLTKDPLA 249


Y.lipolytica_49kD        DIY-MWATQFGDRLDEIEELLTDNRIWKLRTVNIG------------------------- 257
M.barkeri_EchE           MLLKMCDSLET-EIKNIEKVFVNNYTVKQRLVGLA------------------------- 199
D.fructosovorans_LS      NYLALSKEVCQFVNECYIPDLLAVAGFYKDWGGIGGTSNYLAFGEFATDDSSPEKHLATS 309
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Y.lipolytica_49kD        ----------TVTAQDALNLGLSGPMLRGSGIPFDIRKNAPYDAYDKVDFDVPVGMNGDC 307
M.barkeri_EchE           ----------TLSKQVAYEVGTAGPTLRGSGNAIDVRETPDWDIYKDLGFKTAVEKDGDC 249
D.fructosovorans_LS      QFPSGVITGRDLGKVDNVDLGAIYEDVKYSWYAPGGDGKHPYDCVTDPKYTKLDDKDHYS 369

Y.lipolytica_49kD        YDRYLIRMAEFRQSLRIIEQCCN------------DMPAGAVKVEDFKINSPP------- 348
M.barkeri_EchE           YARTKVRITELLNSLTIIRNAIS------------KMPEGEIEVR-VKGFP--------- 287
D.fructosovorans_LS      WMKAPRYKGKAMEVGPLARTFIAYAKGQPDFKKVVDMVLGKLSVPATALHSTLGRTAARG 429

Y.lipolytica_49kD        ----------RNLMKEDMEALIHHFLLYTKGYSVPPGETYTAIEAPKGEMGVYVVSDGSE 398
M.barkeri_EchE           ----------------------------------TGEAIMRTEQPRGEVIYYVKGNGTK 312
D.fructosovorans_LS      IETAIVCANMEKWIKEMADSGAKDNTLCAKWEMPEESKGVGLADAPRGSLSHWIRIKG-K 488

Y.lipolytica_49kD        RPYKCKIRAPGFAHLG---------AFDHIARGHFLPD------AVAIIGTMDLVFGEVD 443
M.barkeri_EchE           KLERLKVRTPTFANIP---------SLLLMLPGVKLAD------VPIVVLTIDPCVSCTE 357
D.fructosovorans_LS      KIDNFQLVVPSTWNLGPRGPQGDKSPVEEALIGTPIADPKRPVEILRTVHAFDPCIACGV 548

Y.lipolytica_49kD        R------------------------------- 444
M.barkeri_EchE           R------------------------------- 358
D.fructosovorans_LS      HVIEPETNEILKFKVC----------------- 564
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