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## SUPPLEMENTARY ONLINE DATA Chloroplast HCF101 is a scaffold protein for [4Fe-4S] cluster assembly

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|   | 102 128  |
|---|--|
| Arabidopsis HCF101                                | : EKDULKA SQIIDEDFGTDEVSCOFVKDGINEALGEVSFREELTPACEVKDMFENKANEVWAAEPWVKKVNUTMSA   |
| Oryza sativa                                      | : KKDVLVALSQIID-DFGTDIVS_GFVKDIEISEALEEVSFRIELTTPACFIKDMFEEKANEVVAALPWVKKVNVTMSA   |
| Populus   | : ESDVLKALSQIID-DFGTDIVS_GFVKDINIDEAQGEVSFRUELTTPACFVKDMFEQKANEVVALLPWVKNVEVTMSA   |
| [35] A. B. M. | : EADVLKALSQIID-DFGTDIVS_GFVKDIQINEALGEVSFR-ELTTPACFIKDMFEQKANEVVAM_PWVKNVNVTMSA   |
|   | : KKDVLGAVSQIID-DFGTDVTCGFVKETVDESTGEVSFQVELTPACPVKDMFEQQAKEKVSA PWVKGVNVKMA   |
| Selaginella                                       | : GHDVLVALSTIID-DFGADIVT-GFVKDIQADKSSGEVAFR ELTTPACFVKDMFEQQAKEKVAALPWVTNVKVTMSA   |
| Volvox  | : EEQULAK RNVID DFGED VACGFVROI EVDASVGFVSFT ELTTPACFVKEMFOROSTOFVKE PWVRDVS KL A  |
| Ostreococcus tauri                                | : ESEVISK RRVID DFGED VN GFVKALVIDESAGSVIFA ELTTPAC VKAEFEROAKAFVEE DWVKRVSVTMA  |
| Micromonas  | : EADVINATENVID-DFGEDTVNCGFVKDIRVSDAGDVTFTTELTTPAC-VKEEFDRLSKQVVTATEWAKSCNVNM-A  |
| Galdieria maxima                                  | : QKQVLEL_KNIED-DLKQNIVELGFVQN-ERVAKEDGKYDVRFT-QLTTPACPIKEKFQNDAKEWVSS-LWVRNVE-DLRA  |
|   | : TEQUESA KAVVD-DLGQD VTLGFVKNI-QFGGDEHYGTVSFDVELTTPACPIKERFREECTRLAES PFVTRANVRLA   |
| Phaeodactylum                                     | : QGEVLST KSVIDPDLGSD VTLGFVQNKLDGRDVSFDVELTTPACPVKEQFQLDCQQLVQD PWTNNIQVTM A  |
| Thalassiosira                                     | : QSQILAA SVIND DLNAD VSLGFVQNKIDESSNIVSLD ELTTPAC PVKDLFVQQ QDI NG AWTRGAD VL S   |
| Chloroflexus                                      | : EDQ LAA ROVQEPELGGD VSRQMVKHAICDGIVRCT ELTTPACPLKDQIRSEAEAAVLAVPGVREVH EF A  |
| Cytophaga   | : QEQVLEA KTVPE DLKKD VTLNM RDAIDGQN SFT VLTTPACPLKELIRNS TEA HK VSGTAVV INMT  |
| Nostoc punctiforme                                | : SRSILEI RPVED ELRKS VELNM RNVKIDGGKVSFT VLTTPACPLREFIVED QKAVKK PGVTDVS EV A   |
| Synechococcus                                     | : AAA LEA RPVQD ELRRS VELNM RDRVEPGRVAFT VLTTPACPLREFIVEECKAA RQ APIEAID TV A  |
| Magnetococcus                                     | : EPQVRDA RMVVD VAGRD VSAGY SGEIHAGE AFQ QFRPESADYLKQLQEQ AQV GA PGVERVT NMSG  |
| Salmonella  | : RAMVAGT ANFQH TIKHN TILKA HHVAWMDDT HVE VMPFVWNSAFEVLKEQ SAD LR TGAKAIDWKL Y   |
| Arabidopsis-L1                                    | :  |
| Chlamydomonas-L1                                  | :MGWRQGAAEWLLSNAASGWRGAASAAALAAGGASGTARAGAPAGASRGA   |
| Homo sapiens                                      | :  |
| Yarrowia lipolytica                               | :MRGFR IAP QRSIAIISRLQPITANFHSSPALRSHENPIGPKSP   |
| Arabidopsis-L2                                    | :MENGDIPEDANEHCPGPQSESAGKSDSCAGCPNQEACAT   |
| Chlamydomonas-L2                                  | :MASSASAAPTGEVPDNANOHCPGTASDQAGKSAACAGCPNQSICAT  |
| Homo sapiens                                      | :MEEV HD PGADSAQAGRGAS QG PNQRL AS   |
| Saccharomyces Nbp35                               |  |
| Drosophila  | :  |
| Homo sapiens                                      | :  |
| Danio rerio                                       | 1  |
| Saccharomyces Cfb1                                | 1  |
|   | 184  |
|   | : QPARPIFAGQLPFGFSRISNIH VSSCKGGVCKSTVAVNLAYTLAGMCARVCTFDADV GPSIETMVNPESRIFEMN  |
|   | : OPARPAYAGELPEG TONISMITA VSSCKCGVCKSTVAVNLAYTLAGMCARVCIFOD DV-CPSIPTAVSPDARLTVMN   |
|   | : OPAKPIFAGOLPFGUSRISNITAVSSCKGGVGKSTVAVNLAYTLAGMCARVGIFDADVYGPSLPTMVNPESRIJEMN<br>: OPARPAYAGELPEGLOKISNITAVSSCKGGVGKSTVAVNLAYTLAGMCARVGIFDADVFGPSLPTMVSPENRLUMN<br>: OPARPVYAGOLPGGLOTISNITAVSSCKGGVGKSTVAVNLAYTLAGMCARVGIFDADVYGPSLPTMVSPENRLIEMN   |
| Vitis vinifera<br>Physcomytrella                  |  |
| Selaginella                                       | : OPAKPLIADDVPAGUKKVSNIVAVSSCKGGVGKSTVAVNLAYSLAQMGARVGIFDADIYGPSLPTMVSPSVKVLOMN<br>: OPAKALAAEGLPRSLONVSNIIAVSSCKGGVGKSTVAVNLAYSLAGMGAKVGIFDADVYGPSLPTMVSPSLRVLOMV<br>: OPPKPLLPESGRPGG-UAKVRHIIAVSSCKGGVGKSTVSVLAYTLAQMGAKVGIFDADVYGPSLPLMVNPSIKVLEMD<br>: OPASNDABF_TVEG-UPSVGHUIAVSSCKGGVGKSTVSVLAYTLAQMGAKVGIFDADVYGPSLPLMVNPSIKVLEMD  |
| Volvox  | : QPPKPLLPESGRPGG-DAKWRHIIAVSSCKGGVCKSTVSVNLAYTLAQMCAKVGIFDADVYGPSIPLMVNPDIKVJEMD  |
| Ostreococcus tauri                                | : OPARNDAPE-TVEGIRRVSHIIAVSSCKSOVKSIVSVILATIAAMCAKVGILDADVYGPSLETMISPDVPVLEMD  |
| Micromonas  | : QPARNDAPE-TVEG - FRWSH: IAVS:CKGGVCKSTTSVNLAYTLAMMCAKVGILDADVYCPS:PTM_SPDVPVIEMD<br>: QPVTNDMPD-AVEG - LKGVRH: IAVS:SCKGGVCKSTTSVNLAYTLRMMCAKVGILDADVYCPS:PTMTSPEQAVLOMD<br>: NEINRAQAGDRPINKWKH: IAVS:SCKGGVCKSTVAVNLAFT_TKLCGKVGIMDADIYCPS:PTMTSPEQAVLOMD<br>: PTSAAAPEAGGSRDPISOVSN:VLVTSSKGGVCKSTVAVNLAFT_TKLCGKVGIMDADIYCPS:PTMTVPSHNEKRIR<br>: OPSVCAFTNSOUGAULIVSSCKGGVCKSTVAVNLAFT_TKLCGKVGIMDADIYCPS:PTMTVPSHNEKRIR   |
| Galdieria maxima                                  | : NEINRAQAGDRPPNKVKHUIAVASCKGGWGKSTVAVNLAFTLTKLCGKVGIMDADIVGPSHEIIVQPSNKIVQYK  |
| Cyanidioschyzon                                   | : NEINRAQAGDRPDNKVKHITAVASCKGGVCKSTVAVNLAFTITKLCGKVGIMDADIYGPSLPILVQPSNKIVQYK<br>: OTPSAAAPEAGGSRDPLSOVSNIVLVTS <mark>AKGGVAKST</mark> TAVNLAFVLARLCARVGILDADIYGPSLPIMVNPBHNEKRIR  |
| Phaeodactylum                                     | : OF SAMAP BAGGS REFLOWS. ULTISANS WARS TAVIDATE WARS AN OUT DADITION THAT AND AND THE SAME STATE OF S |
| Thalassiosira                                     | : QPTAAPSDAPLGMSQIGAVIAVSSCKGGVGKSTTAVNLAFADESLGAKVGIFDADVYGPSDPTMVTPDDDNVRFV  |
| Chloroflexus                                      | : QPTAAPS - DAPLG - MSCIGAVIAVSSCKGGVCKSTTAVNLAFALESLCAK - VGIFDADVYGPSIPTMYTPBDDNYFFY<br>: NVRRPAGIPEQSA - IPGYAN IAVAACKGGVCKSTVANLAVALAQMCAQ - VGLDADVFGPSIPTMYDPDDNYFFY<br>: ADVTTGRPNSGPV - IPHYKN IAVSSCKGGVCKSTITANLAVALSKSCAK - VGIDADISGPSIPTMFDVDVPNVI<br>: ETPQCKSLPDRTG - ISGYKNIIAVSSCKGGVCKSTVAVALAQTCAK - VGLDADISGPSIPTMFDTMDDDAQTVR<br>: ETPRSPSLPNRQS - IGOVRNIIAISSCKGGVCKTSVSVNAVALAQSCAR - VGLDADIYGPN  |
| Cytophaga   | : ADVTTGRFNSGPVUPHVKNIIAVSSCKGGVGKSTITANLAVALSKSCARVGIIDADISGPSIPTMFDVDVPNVI   |
|   | : ETPQQKSLPDRTGISGVKNIIAVSSGKGGVGKSTVAVNVAVALAQTCAKVGLLDADIYGENDPTMUGLADAQUVVR   |
| Synechococcus                                     | : ETPRSPSLPNRQSHPGWRNIIAISSGKGGVGKTSVSVNVAVALAQSGARVGLLDADIYGPNVPLMIGLODRSLVVQ   |
| Magnetococcus                                     | : NPQQQAEPL  |
| Salmonella  | : NIATLKRVKNOPGHNGVKNILAVSSGKGGVCKSSTAVNLALALAAEGAKVGVLDADIYGPSIPTMLGAEDORPTSP   |
| Arabidopsis-L1                                    | : YKFSSASAGGRTTELRUHGVKDIIAVASGKGGVGKSSTAVNLAVALANKCELK-IGLLDADVYGPSVPIMANINQKPQVNQ  |
| Chlamydomonas-L1                                  | A A SRGA A A A GPOKKI, GIK DWOHING IT IS A K COW CKSINT AND A VALUE TRI, GLR - WOHIND WHOLS IN STRUCTURE OF FLDK   |
| Homo sapiens                                      | : QRRTQIMSRGLPKQKPIEGVKQVIVVASGKGGVCKSTTAVNLALALAANDSSKAHGLLDVDVYGPSVPKM/NLKGNPELSQ  |
| Yarrowia lipolytica                               |  |
| Arabidopsis-L2                                    | : -APKGPDPDLVAIAERASTVKHKILVLSGKGGVGKSTFSAQ SFALAGMDHQVGLMDIDICGPSIPKM GLEGQETHQS  |
| Chlamydomonas-L2                                  | - APKGPDPDLAAIAARMSRWKHKULWLSGKGGWCKSWVSAOLAFALARREFE - WGUUDIDTCGPSVPKWUGLDGOETHSS  |
| Homo sapiens                                      | : GAGATPDTAIEEIKEKMKTVKHKTIVLSGKGGVGKSTFSAHLAHGLAEDENTQ-IALLDIDICCPSIPKIMGLEGEQVHQS  |
| Saccharomyces Nbp35                               | A DECEMBER AND A DECEMBER AND A DECEMBER AND AND AND A DECEMBER AND A  |
| Drosophila  | :vglldidlegpsvgyllglegrdifqc   |
| Homo sapiens                                      | :meaaaepgnlagvrhiilvlsgkggvgkstistelalalrhagkkvgildvdlcgpsiprmlgacgravhqc  |
| Danio rerio                                       | :MDGSGKGNDDQVKHVLUUSGKGGVGKSTITTELALAFRHACKKVGILDVDLCGPSIPRMLSVGKPEVHQC  |
| Saccharomyces Cfb1                                | :MEEQEIGVPAASLAGIKHIILIISGKGGVGKSSVTTQTALTICSMGFKVGVLDIDLTGPSLPRMFGLENESIYQG   |

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|  | 303   |
|--|---|
| Arabidopsis HCF101   | : PEKKTIIPTEYMGYKLVSFGEAGQGRAIMRGPMVSGVINOLITTTEWGELDYLVIDMPPGTGDIQLTLCQVAP   |
| Oryza sativa   | : PESRSILPTEYLGVKMVSFGBAGQGRALMRGPMVSGVINGLLTTTDWGELDYLVIDMPPGTGDIHLTLCQVAP   |
| Populus  | : PEKRTIIPTEYLCVKLVSFGFAGQGRAIMRGPMVSGVIDOLLTTENGELDYLVIDMPPGTCDIOLTLCOVVP<br>: PEKRSIIPTEYLCVKLVSFGFAGQGRAIMRGPMVSGVINOLLTTENGELDYLVIDMPPGTCDIOLTLCOVVP  |
| Vitis vinifera<br>Physcomytrella   | : PEKRSIIPTEYLCVKUVSFGBAGQGRAIMRGPMVSGVINOLITTEWGELDYLVIDMPPGTGDIOLITCOVVP<br>: PETRAIIPTEYLCVKUVSFGYAGQGSAIMRGPMVSGVINOFLTTDWGELDYLVIDMPPGTGDIOLICCVVP   |
| Selaginella  | : EDTKQIIPTEYLGVKLVSFGFAGQGTAIMRGPMVSGVINQLLTTTDWGELDVLIIDMPPGTGDIQLTLCQVVP   |
| Volvox   | : PATKAIFFTEYEGVKVVSFGFAGQGSATMRGPMVSGLIQOMLTTAANGELDYLVVDFPPGTGDIOLTLCOTVS   |
| Ostreococcus tauri   | : KETGTIKPVEYEGVKVVS <mark>F</mark> GBAGQGSALM <mark>RG</mark> PMVSGLINOLLTTTD <mark>WGELDYLLIDMPPGTGD</mark> VQLTLCQVVP  |
| Micromonas   | : KETGSITPTEYEGWGIVSFGEAGQGSAIMRGPMVSGLINOMLTTTAWGDDVLIIDMPPGTGDVQLTICOVLP  |
| Galdieria maxima<br>Cyanidioschyzon  | : DGRIIPLEYENVKIMSPGYINPESAIMRGPHIANMINOLITETDWESEDYLVIDMPPGTEDIOLITICOTVS<br>: LTPDGLMVPLTRAGVKIMSPGYINSDPAVLRGPMVSSLITOLIOOTDWESEDYLLVDIPPGTEDIOITIGOVIK  |
| Phaeodactylum  | : GROVAPLORNGWRLMSFGYVNDGSAVMRCPMVTOLLDOFLSVTHWCALDVLTUDMPPGNCDTOFTUTOKUN   |
| Thalassiosira  | : GRQHAPLERGDVSLMSFGYVNEGSADMRGPMVTQLLDOFISLTNWCAHDYLIMDMPPGTGDIOLTISORLN   |
| Chloroflexus   | : DANGOPM-MIPLISHHCHKVMSVCFID-ESOPVWRCPMVSOF ROFIYOVANAPHDVITHDVPPCTCDVALUASIP  |
| Cytophaga  | : ENENGKPTHIPIDQYCVKIISIGFISP-AESAVVWRCPMASSARCFISDCDAGEDDYHFDMPPGTSDIHEHVQTVP  |
| Nostoc punctiforme<br>Synechococcus  | : STETGDI-LEPAFNHGVKLVSMGFLID-RDOPVLWRGPMLNGVLROFLYQVQWGELDYLIVDMPPCTGDAGLTLTOAVP<br>: KREDGGEDIFFLENYGVKVVSMGLUVG-RDOPVLWRGPMLNGVLROFLYQVQWGELDYLIVDMPPCTGDAGLTLVOAVP  |
| Magnetococcus  | : EKGQKVT:M:KYGVKINSMG5FMP-EDTPMIWRGPNVGMAVECLIRDIDWGSLDYLVIDLPPGTGDA0LTITCKVP  |
| Salmonella   | : DGTHMAFIMSHCLATNSICYLVT-DDNAMVWRGPMASKALMOMLQETLWPDLDYLVIDMPPGTCDIOLTIAON P   |
| Arabidopsis-L1   | : DMKMIPVENYCVKCNSMCL-VE-KDAPLVWRGPMVMSALAKMTKGVDWCDLDILVVDMPPGTGDAOISISON-K<br>: SGTGALMLPKENYRVKTNSFCFFLE-GDEPVVWRGPMVNNAFDKM-FGTEWCLLDVLVVDMPPGTGDAOIN-GCR-P   |
| Chlamydomonas-L1   | : SGTGALMLPKENYRVKTMSFGEFLE-GDEPVVWRGPMVNNAFDKM/FGTEWCLLDVLVVDMPPGTGDACIN/GCRIP   |
| Homo sapiens<br>Yarrowia lipolytica  | : SNLKPLLNYCHACKSMGFLVE-ESEPVVWRGLMVMSALEKLIRQVDWGQLDVLVVDMPPGTGDVQLSVSONIP<br>: EGKLIPMSKFCHQVMSMGFLVD-PNKAVAWRGLUVQKALECIYQDVDWGTLDVLVMDIPPGTGDVQLTIACTIK   |
| Arabidopsis-L2   | <ul> <li>NLGWS PU/VMODN IGU/AST(elo)/IEDNSDEAM/IM/Jelo/RKNG/IE/KO/V//////////////////////////////////</li></ul>   |
| Chlamydomonas-L2   | : GAGWSPVYVEDNPAVMSIGFMLPNPDEAVIWRGPRKNGLIKOFLKDVDWGELDYLVVDAPPGTSDEHITITOCLO   |
| Homo sapiens   | : GSGWSPVYVEDNWGVMSVGETLSSPDDAV/WRGPKKNGMIKOFIRDVDWGPVDVLTVDTPPGTSDEHUSVVRYLA   |
| Saccharomyces Nbp35  |   |
| Drosophila<br>Homo sapiens   | : NSGWIpvrHubDATSLOT DPEDUSALWRSKNALD KKT KUMMOND HVIDTDPOISDBHINKSK<br>: ddgwpvrHubthrigiflknredpvikrgpkkmalikafvsdvawgeldylvvdtopgtsdehitvmelk<br>: drgwapvfldre-qsiglmsvofllekpdeavvwrgpkknalikafvsdvawgeldylvvdtopgtsdehmaticalr  |
| Danio rerio  | : DSGWVPVYADPQQQQDALMSIAFILEDSDEAVIWRGPKKTALIGOFVSDVAWGELDILLVDTPPGTSDEHDAVLENLR  |
| Saccharomyces Cfb1   | : PEGWQPV-KVETNST-GSESVISLGFLLGDRGNSVIWRCPKKTSMIKOFISDVANGELDVLLTDTPPGTSDEHTSIABELR   |
|  | 339 347   |
|  | : LTAAVIVTTPOKLAFIDVAKGVRMES-KLKVPCVAVVENMCHFDADGKRYYPCKCSGSEVVKQFGIP<br>: LTAAVIVTTPOKLAFIDVAKGVRMES-KLKVPCVAVVENMCYFDADGKRFYPCQCSGAQVVQQFGIP  |
| Oryza sativa<br>Populus  | : LTAAVIVTTPOKLAFIDVAKGVRMESKIKVPOVAVVENMOYFDADGKRFYPFGQCSGAQVVQQFGIP<br>: LTAAVIVTTPOKLAFIDVAKGVRMESKIKVPOVAVVENMOHFDADGKRYYPFGRCSGSQVVQQFGIP  |
| Vitis vinifera   | : TADADGKRYYPECRESGSOVVOOFCP  |
| Physcomytrella   | : LTAAVIVTTPOKLAFIDVAKGVRMESKIKVPCIAVVENMCFFEGDDKRYYPEGKCSGSKVVEQFGIP   |
| Selaginella  | : LTAAVIVTTPOKLAFIDVAKGVRMESKIKVPOLAVVENMCYFDADGKRYYPFGRESGKQVVQQFGIS   |
| Volvox<br>Ostreococcus tauri   | : FSAAVIVTTPCKLAFIDVAKGIRMFA-KIVVPCVAVVENMSYFEADGKRFFPFCQCSGERIQRDFGLP<br>: ITAAVVVTTPCKLAFIDVEKGVRMFA-KIAVPCVSVVENMSYFEVDGVKHKPFCECSGAKIGEQYGVP  |
| Micromonas   | : ITAAVVVTTPQKLAFIDVBKGVRMESKIRVPCVAVVENMSYFDGDDGKRYKPFGECSGQRICDDYGVP  |
| Galdieria maxima   | : LDAAVIVTTPOOLSFODVIKGIOMFGKVSVPCVALVENMAYFEPNDIPDKRYYLFGHCKSOKIANDYGIP  |
| Cyanidioschyzon  | : ATAAVVVTTPORDAFADVVRGTOLLDRVAVPPTAVVBSVATFVAPDTGKRIDLFGKCHSARISREFGIR   |
| Phaeodactylum  |   |
| Thalassiosira<br>Chloroflexus  |   |
| Cytophaga  | : VTGATVVTTPCKVATADAORGI OMEROPOVNV PVIGVTENMAWET-PARLPENKYYTECKDGGKETAEKEDVP   |
| Nostoc punctiforme   | MAGAVIVINPOTWALLDSRKGERMEOOMNUPULGEVENMSYETPPDOPDKHYDTECSCGGSKTAAELGVP  |
| Synechococcus  | : #AGAVIVTTPCSVALHDSRKG#NMERQHGVPIFGIVENMSYFIPPDLPDRQVDIFCSEGGETTARELGVP  |
| Magnetococcus  | : LSGVVIVSTPODVALADVRKGINMFKKVEVPVIGIIENNSYNDCTECGHRAEIPSHEGAEKEAANSONT   |
| Salmonella<br>Arabidopsis-L1   | LISCAVTVSTPODVALADANEGISMED   |
| Chlamydomonas-L1   | : LSGAALVSTPQDVALIDVRRCAQMFLKLRVPLLGLIENMAYHRCGKCGHVEHIFCTGGVERAAADYGMD   |
| Homo sapiens   | TTGAVTVSTPODIALMDAHKCAEMERRVHVPVLGLVONMSVEOOPKOKHKTHTECADGARKLAOTLGLE   |
| Yarrowia lipolytica  |   |
| Arabidopsis-L2<br>Chlamydomonas-L2   | PIGIDGALIVITPOEVSLIDVRKEVSFKKVGVPVLGVVENSGLSOPLLDIVACSEVFDSSG GAERACREMGVP<br>VGGLGGGAAAVIVTTPODVAIIDVRKEVNFCKVGLPVLGVVENMAGLRFPGAQLRLRAEVFRE-GGAARACADMGVP<br>TAHIDGAVIITPOEVSLQDVRKEVNFCRKVKLPIIGVVENMSG IOPKCKKE-SQIFPPTT GAELACQDLEVP<br>E SGIDGALVVTTPOEVSLQDVRKEVDFKKAGUNILGIVENMSG VOD KKE-SQIFKATTGGGALCKELTK<br>AVKCDA   |
| Homo sapiens   | TAHIGGAVIITTPEOVALDUKASUAPRE-KUKASUAPITGVISINAGENFOAQLABARVASUAPOOLEV-  |
| Saccharomyces Nbp35  | : ESGIDGALVVTTPOEVALLDVRKETDFCKKAGINILGLVENNSGFVCPNCKGESQIFKATTOGGEALCKELGIK  |
| Drosophila   | : evgchgaivttpgevildvrkeitfckktginilgivenmsgivcphctsgtnifssnggvslatyaqvp<br>: pyqplgalvvttpgavsvgdvrreitfcrktgirvmgivenmsgitcphctectsvfsrgggeelaqlagvp  |
| Homo sapiens   | : pyqplgalvvttpqavsvgdvrfeltfcrtglrvmgivenmsgitcphotectsvisr_ggeelaglagvp<br>: KHRVDGAVLVTTPQAVSTGDVRREITFCKKTNIKILGVVENKSGVCPHCSECSNISKCGGEELAKLTCSA   |
| Danio rerio<br>Saccharomyces Cfbl  | : KHKVDGSULVIVICAUSTGOURNEITERINUTIDOUSINGGUCHESSGSULISK GGEE ALLISK<br>: YSKPDGGUVVITPCSW.TADUKKEINFCKKVDIKIEGIISINSGUCPHCAEGTNI-SSCGGKRISEQFSVP   |
|  | 414 419   |
|  | : HEFDERETESASGDSCTPEVVSDEL-SDVARTEQDEGVCVVQQCAKIRQQVSTAVTYDKYLKAIRVKVP   |
| Oryza sativa<br>Populus  | : H FD PIRT SASCIT I EVVAD Q-GDV KT QN GVCVVQQAKIRQQVSTAVSYDRSIRAIRVKVP<br>: H FD PIRT SASCIC M EVAD Q-GEV KI QN GICI QQAKIRQQVSTAVTYDKSIKAIKVKVP   |
| Vitis vinifera   | : HIFD PIR TISASG SCH BUARD COSDING OF CONCERNMENT OF STORE STO |
| Physcomytrella   | : HIFE DIREISAAG TON EVVVD Q-GQVANIESD VGVOVQOCAKLRQAVSTAVMYDKAINAIRVKVP  |
| Selaginella  | : NIFEFPIRPEARLYKALSAAGISGTPEVVHDPQ-GDVARSPSEIGVCVVQQCAKIRQQVSTAVTYDDAMRAIKVKVP   |
| Volvox   | : NUVEPEIVEDUSAGDGCOPLIVADET-SATAARMDLGAAVVREVAKMAGRPARQAVYDPQKDVISVQLP<br>: NULQYPIVEDUSAGDTCRPLVLRDET-CETSSRYQEVAATVVREVAKLNNGKKPRVDIDPGYDGAFRVEIP  |
| Ostreococcus tauri<br>Micromonas   | : N LQ PIVPD SA GOT RPLVERD T-CETSSRYQEVAATVVREVAKLNNGKKPRVDIDPGYDGAFRVEIP<br>: N FQ PIVPD SA GOT RPLVEVD A-GDVSTIYGAVAAKVVQEVAKLQAGPKGSLALDTEGVAGVDGA  |
| Galdieria maxima   | : FVESFPLDPD_CRWSDNCIPAVLALSE-SKISQLYQSPASAVVQQIAKNAFGN-GKRIPQVFFDSDKCIIVISCN   |
| Cyanidioschyzon  | : STFQVPTWEENAAGDTCTPVTITLPETSEIFQCVRRIAENIVQEGARVRFG-AVPIPQARWDADHREIVVQLQ   |
| Phaeodactylum  | : HSFSIPHLNKUAANGDNGTEFVLEFED-SPPAKIYQEDASAVVSEVAKTKFAKSMRPSVQYDAESHLLQVSQN   |
| Thalassiosira<br>Chloroflexus  | : HTYS YPLMGOTAQNG SCT FIIDNEK-SPQADIYRQ AKSVYSEVAKIKFCTGKGGRPSVSYDVEKSILRVDDG<br>: V GQ PPLGMSVREGG N Q AVISDAP-DAYADI RELARQ VARISVLQYAMV   |
| Cytophaga  | : LI GQIPLVQGIRESG-MCKPAVIN-LD-KIT-QA-KELAETVAQQVAIRNASLAETRKVEIKV  |
| Nostoc punctiforme   | : L GCVPLEISTRVGGDSGVPIVVGDPD-SASAKALTAIALTIAGKVSVAALT  |
| Synechococcus  | : LIGRIPIEIAURQGGDACQUIVISQUE-SASUQALRQUAKTUAGRVSMLALGAG  |
| Magnetococcus  | : FIGHIPISEDIRKDSDACKPIVVARPD-SPQ.QQVLEIARNVVSKLQDGAGAPKMPKIVIE   |
| Salmonella<br>Arabidopsis-L1   | : L GQ YELHIS REDL R TITIVSR E-SEFTAIYRE ADR AAQLYWQGEVIPGEIAFRAV   |
| Chlamydomonas-L1   | : VIGQVERHVDIQTRSDACT:VVASSGGSIVSAAQD AQAVAGIABLEREN-PDABIQMALAVPHSSHSS   |
| Homo sapiens   | : VLGDIPLHLNIREASDIGQPIVFSQPE-SDEAKAYLRIAVEVVRRLPSPSE   |
| Yarrowia lipolytica  | : VIGNUPLDEQICSQSDK_VPVAVSGGVQAKYYDKIAEGVAEQIGV   |
| Arabidopsis-L2   | : FLGKVPMDFQLCKAAEQCKSCFEDNKC-LIS PALKS IQKVVPSTVMTE  |
| Chlamudamana = = 0   | TI OPI DI DI CANADA DOM DENACANO DAVIZO DAVIZO DAVIZ  |
| Chlamydomonas-L2<br>Homo sapiens   | : LUGRUPTDGUGAADA CRSV PEAAG-AAGADAVKGGLEGVAP   |
| Chlamydomonas-L2<br>Homo sapiens<br>Saccharomyces Nbp35                          | : L GRVPIDL GKNCCK QSFF DA D-SPATLANRS IQR QEFONLHQSKEENLISS  |
| Homo sapiens<br>Saccharomyces Nbp35<br>Drosophila                                | : L GRVPLDL GKNCFK QSFF DA D-SPATLANRS IQR QEFONLHQSKEENLISS  |
| Homo sapiens<br>Saccharomyces Nbp35<br>Drosophila<br>Homo sapiens                | : L'GRVPLD:L'GKNCCK OSFFIDA D-SPATLAVRS IQR QEFONLHQSKEENLISS   |
| Homo sapiens<br>Saccharomyces Nbp35<br>Drosophila<br>Homo sapiens<br>Danio rerio | : L GRVPLDL GKNCFK QSFF DA D-SPATLANRS IQR QEF NLHQSKEENLISS  |

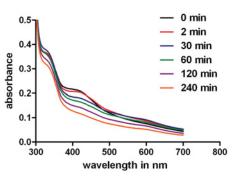
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| Arabidopsis HCF101 : NSDEEFLLHPATVRRNDRSAQS-VDEWTGEQKVLYGDVAEDIEPEDIRPMGNYAVSITWPI<br>Oryza sativa : DSDEEFLLHPATVRRNDRSAQS-VDEWTGEQKVQYGDIPEDIEPEEIRPMGNYAVSITWPI | GFSOIAPYDOL |
|--|-------------|
| Oryza sativa : DSDEEFLLHPATVRRNDRSAQS-VDEWTGEQKVQYGDIPEDIEPEEIRPMGNYAVSITWPL   |             |
|  |             |
| Populus : DSEEEFLLHPATVRRNDRSAQS-VDEWTGEQKLQYADVPEDIEPEEIRPMGNYAVQITWPI  |             |
| Vitis vinifera : DSEEEFLLHPATVRRNDRSAQS-VDEWTGEQKLQYADVPEDIEPEEIRPMGNYAVSITWPI   |             |
| Physcomytrella : GTTEEFLLHPATVRRNDRSAKS-IDEWSGEQKLRYTDVAEDLAPESIRPMGNYAAAINWPI   |             |
| Selaginella : GTEEPFYLHPATVRRNDRSAKS-IDEWTGEQKLRYGDVREDIEPEAIQPLGNYAVMISWPI  |             |
| Volvox : GETEFLLPPVVVRENDTSATS-IDEWTGQRK-RDEVPQDARPAAINPLGNYAVQISWSI   | GFNQVASYELL |
| Ostreococcus tauri : GENNDKAFWITAKNVRLSDESARVKGSDESPDRLLNGAPIPDDIAPVEMSVIGNYAMSITWP  |             |
| Micromonas : LRVQLADEGGMPFYVRGCDVRRSDKSATA-DGESKKADFLMDGVTPVPDDIAPVEAHVVGNYAVQISWPI  |             |
| Galdieria maxima : DQQGIAWNENNKVEWSPWELRNACSCASC-VDEFTGKRHWKSVDRNVKPLQIQTAGNYAFSVIWSL  | GHQSLYPFERV |
| Cyanidioschyzon : DHMEERIQPAALRACROAAC-VDECTGKQLLDPNSVDDNIYPMQMMNVGNYALAVNWSI  |             |
| Phaeodactylum : GVGSTDEEHVATLPPAELRRACCAAC-VEELTGRQILVPSSVSDKIAPRNMVPTGNYALSVDWSI  |             |
| Thalassiosira : DIQNATISPAELRRGCRCAAC-VEELTGKQILNPASISESVKPLNMSPTGNYALSVDWSI   | GHRSLYPYRQI |
| Chloroflexus :   |             |
| Cytophaga :  |             |
| Nostoc punctiforme :   |             |
| Synechococcus :  |             |
| Magnetococcus :  |             |
| Salmonella :   |             |
| Arabidopsis-L1 :   |             |
| Chlamydomonas-L1 :   |             |
| Homo sapiens :   |             |
| Yarrowia lipolytica :  |             |
| Arabidopsis-L2 :   |             |
| Chlamydomonas-L2 :   |             |
| Homo sapiens :   |             |
| Saccharomyces Nbp35 :  |             |
| Drosophila :   |             |
| Homo sapiens :   |             |
| Danio rerio :  |             |
| Saccharomyces Cfb1 :   |             |

## Figure S1 Multiple sequence alignment of the FSC-NTPase family

Representative protein sequences of different organisms are shown subdivided according to the proposed four FSC-NTPase classes [23]. Organism names are coloured according to the class affiliation. Green background represents plants and algae taxa belonging to the class I. Yellow background represents eubacterial class I proteins. Grey background corresponds to class II members. Blue and magenta congregate taxa belonging to the class IV respectively. Note that algae and plants class I proteins have a C-terminal extension corresponding to the DUF971 domain (COG3536). The very N-terminal and C-terminal regions are not shown in the alignment as they show little conservation. Amino acids coloured with a black background are 100 % conserved. Amino acids coloured with a grey background and with white characters are > 60 % conserved. Amino acids coloured with a grey background and written with black characters are > 40 % conserved. Cysteine residues conserved in at least two sequences are depicted with a blue background. Numbers above the conserved cysteine residues in plants and algae correspond to the position of the residue in the sequence of the *Arabidopsis* HCF101 protein.

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## Supplementary Figure S2 Oxygen sensitivity of the reconstituted HCF101 protein

Purified HCF101 protein was reconstituted as described in the Experimental section in the main paper. Decay of the shoulder at 420 nm in the presence of oxygen was monitored by UV–visible spectroscopy.

## Supplementary Table S1 Oligonucleotides used for site-directed mutagenesis and cloning

For details please see the Experimental section in the main paper. for, forward primer; rev, reverse primer.

| Designation | Sequence                                       |
|-------------|--|
| C102S-for   | 5'-GGAGCTGACAACACCCGCATcTCCAGTCAAAGAC-3'       |
| C102S-rev   | 5′-GAAACAATATCTGTCCCAAAATCAGGATC-3             |
| C128S-for   | 5'-CATCATCGCTGTTTCTAGTTctAAGGGTGGTG-3'         |
| C128S-rev   | 5'-GTGTTGTCAGCTCCAAACGGAACGAAACCTC-3'          |
| C184S-for   | 5′-GATATACAACTGACCTTAT <i>ct</i> CAGGTTGCGC-3′ |
| C184S-rev   | 5'-CTAGAAACAGCGATGATGTTCGAAATTC-3'             |
| C303S-for   | 5'-CTCAAAACTTAAGGTGCCTTctGTTGCTGTTGTG-3'       |
| C303S-rev   | 5′-TAAGGTCAGTTGTATATCACCAGTTCCAG-3′            |
| C339S-for   | 5'-CTCAAAACTTAAGGTGCCTTctGTTGCTGTTGTG-3'       |
| C339S-rev   | 5′-GCACCTTAAGTTTTGAGAACATCCTTACAC-3′           |
| C347S-for   | 5'-GCTGTTGTGGAGAATATGTctCACTTTGACGC-3'         |
| C347S-rev   | 5′-CATATTCTCCACAACAGCAACGCAAGGC-3′             |
| C414S-for   | 5'-CGTTCCAGGATCTTGGTGTATcTGTAGTGCAAC-3'        |
| C414S-rev   | 5'-CACCAAGATCCTGGAACGTTCTGGCAACG-3'            |
| C419S-for   | 5′-GTGTATGTGTAGTGCAACAATctGCCAAGATAC-3′        |
| C419S-rev   | 5'-TGTTGCACTACACATACACCAAGATCCTGGA-3'          |
| INCORP-for  | 5'-GTAGAATTCTCAGCTCAAGCTAGTAGTAGTGTTGG-3'      |
| INCORP-rev  | 5'-GTAGAATTCGACTTCGACTGGAGACAATGGAGG-3'        |

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