Colored PROMALS3D alignment (sequences in aligned order)

```
Conservation:        9       7756                                                  6
NRDE2_Dme        1   MS-----LFPAYGAG-------------------------------------------------RTS   13
NRDE2_Hsa        1   MA-----LFPAFAG-------------------------------------------------LSEA   13
NRDE2_Dre        1   MA-----LFPSFSG-------------------------------------------------LSGS   13
NRDE2_Mmu        1   MA-----LFPAFAD-------------------------------------------------VSEA   13
NRDE2_Gga        1   MA-----LFPAFAG-----------------------------------------------APEPGEA   16
NRDE2_Cel        1   -------MFRAYGNNGLKNPERISGENPDLYTQTRAAVQQRATTTLKRNEKQKLAVQNDSVFQQVGIGES   63
NRL1_Spo         1   MPSNHNTSVPKFS-------------------------------------------------------   13
Consensus aa:        Ms.....hFPt@ts.....................................................pt
Consensus ss:

Conservation:                                    6  6 6
NRDE2_Dme       14   AAGS----------AKQTTKPGEEEPSTGGNWKKNESYI------------------------------   42
NRDE2_Hsa       14   PDGG----------S--SRKE--------LDWLSNPSFCVGSITS------------------------   38
NRDE2_Dre       14   NSSA----------ESKAPAD--------LEWLSNQSFSTEDALK------------------------   40
NRDE2_Mmu       14   SGDG------------AFRKE--------LDWLSNPSFRVGNLTS------------------------   38
NRDE2_Gga       17   PAGS----------SEGSRKE--------LDWLSNPSFSTEDALL------------------------   43
NRDE2_Cel       64   DSDDDNGGVRIRMSPHRYIDP--------DDVFTLPEVKKQNALRDAKIAARAAQATAYNTFPSVKSLNG  125
NRL1_Spo        14   ------------------------------------------------------------SFNSVK----   19
Consensus aa:        stss..............c..........shbp..ph.............................
Consensus ss:                           h        hh     hhhhhhhhh

Conservation:
NRDE2_Dme            ------------------------------------------------------------------------
NRDE2_Hsa       39   ------LSQQTEAAPAHVSEGLP-----LTRSHLKSESSDESDTNKKLKQTSRKKKKEKKKKRKHQHHKK   97
NRDE2_Dre       41   ------THQRATDRARAETE---------------EPAGTSEEKHKERGDNHGKSKKRKKEKKKKH--K   86
NRDE2_Mmu       39   ------LSRQTEEVTALASEGSPPPRYSFIRSPLKSELSGESNTSEKLAQTSRKKKKEKKKRRKHQHHRK  102
NRDE2_Gga       44   ------LHQRTTEAANLTPEKSP-----LIRSTSRSDLSGESDTDESLKKSS---KKRKKKKKKHHHYKT   99
NRDE2_Cel      126   CQDPPETSQQSTSRKRSASNSRS-----PSRSHSRR----YDRDNGRQRSRSREKKRRKKERRR------  180
NRL1_Spo        20   ------------------AKKNP-----ITKSN-----------------------------------KK   31
Consensus aa:        ..................sp.................................................
Consensus ss:              hhh hhhh                              hhhhhhhhhhhhhhhhhh

Conservation:         6       57        7        6          6                          7 6
NRDE2_Dme       43   --KKHV-NIATQGPESDSSSSTE-DSEDEA------DKRKQDDVTKTKNVH----------RGKTL----   88
NRDE2_Hsa       98   TKRKHG-PSSSS------RSETDTDSEKDKPSRGVGGSKKESEEPNQGNNA----AADTGHRFVWLEDI-  155
NRDE2_Dre       87   KKRSRD-NSESS------GFESDTIYPSDLL-------KKE-------NTDREEAQVRVSETFMWLDDL-  134
NRDE2_Mmu      103   TKRRHE-QLSSS------GSESDTEAGKDRASRSIRDDQKEAEKPCQGSNAAAAVAAAAGHRSIWLEDI-  164
NRDE2_Gga      100   KKKAKG-DSSSS------ESDLDTKCIKDKAA------AKQAQFPLEFSLD-DKTSNLTSNRSVWLDDV-  154
NRDE2_Cel      181   -KRSSSRSSSSS------RS-------RDRSSRARDTSSHTLMKMN-----------KPAKYAFLTDEE  224
NRL1_Spo        32   YRSSHD-QVSSN-------------------------------------------HAKSSFPSH--    51
Consensus aa:        .++p+s.p.tSs.......S.......c-.........p+p.....................+..@Lpc..
Consensus ss:         hhh                     hhhhhhhh  hhhhhhhhhhhh                ee

Conservation:                   9  7 5    7   6   6   6  67     6  9      6           66
NRDE2_Dme       89   ----EFDSSDGFYVDKVGNSSYRNIKTLKKPARP--RYKTRM--------------------------  124
NRDE2_Hsa      156   ----QAVTGETFRTDKKPDPANWEYKSLYRGDIA--RYKRKGDSCLGINPK-KQCISWE-GTSTEKKH-S  216
NRDE2_Dre      135   ----QTPTDSPFCIDRRADRANWQYKSLYRGDIA--RYKRKGGSCLGLDLR-TQAVTWT-DGGPEKKR-V  195
NRDE2_Mmu      165   ----HDLT-DVFRTDKKPDPANWEYKSLYRGDIA--RYKRKGDSCLGINPK-KQCISWE-GASAAKKH-S  224
NRDE2_Gga      155   ----QAFTTETFRIDKKSDPANWAYKSLYRGDIARYRYKRRGESCLGLDTK-KQCIAWD-SPASEKKQ-L  217
NRDE2_Cel      225   YRTCDAYISSAFITQTKSDCENYTQG-VPKKEIA--KCRLSVKFIVGLEHN-NILFNNIYGAEYARDK-E  289
NRL1_Spo        52   -----RSIQSNFAVDTKGEKQNLLYG-INKRPVP--KYHRSSSSVYGSAPLLRIVKESKEGITLNKKKSL  113
Consensus aa:        ....p..hspsF.hDp+t-..Nh.h..l.+.sls..+Y+ppsp.hhG.....pbhbp...t....+c+..
Consensus ss:                eeee    hhhhhe           hhe     eee      eeee     hhhh

Conservation:                9                              6    7                    966 6
NRDE2_Dme      125   --------KRLGDGKYNP----------------------HDFSRAHS--------------------  142
NRDE2_Hsa      217   RK----QVERYFTKKSVGLMNIDG---VAISSKTEPPSSEPISFIPVKDLEDAA-PVT--TWLNPLGIY-  275
NRDE2_Dre      196   DK----KAERYFSSSTRQLLQTRGLLALPILSEGSATNSDPYIKLAVCAEEQGSSTQTPESWVNPLGIY-  260
NRDE2_Mmu      225   HR----HLERYFTKKNVGLMRTEG---IAVCSNPEPASSEPVTFIPVKDSAEAATPVT--SWLNPLGIY-  284
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NRDE2_Gga      218  QR----RPERYFSKSNVKTLNSDG---IPVCNKSSP--SDPAAFIPVLHVETSDPSDT--TEVNPLGIY-  275
NRDE2_Cel      290  NRPFWEQLDRYLKDV---------------------PKETFFR-----------------YVPPVGGYW  320
NRL1_Spo       114  EIK--YDEERSFDEKEND-------------------ES---EF----------EDGQ--QGFIPLLVN-  146
Consensus_aa:       p.....p.-Rhhspp.s.....................p..h.F.................h.Pl....
Consensus_ss:             hhhhhh

Conservation:             6      7                                  7       77      6
NRDE2_Dme      143  -------KFSLKRSRYVRE-----------------KLIADAPSEDEVSRLQEQLIRTKVLVQQEPEVL  187
NRDE2_Hsa      276  ---DQSTTHWLQGQGPPEQESKQPDAQPDSE------SAALKAKVEEFNRRVRENPRDTQLWMAFVAFQD  336
NRDE2_Dre      261  ---DPGTTLWLEGKGQPEVKGEQQSTEAQESN-----STLLSARVEEFNRNLRENPTDIKLWLDFVHFQD  322
NRDE2_Mmu      285  ---DQSTTQWLQGQGPAEQESKQPDSQQDRE------NAALKARVEEFNRRVRENPWDTQLWMAFVAFQD  345
NRDE2_Gga      276  ---DPSTTEWLQGKGYKGEEHEPVNTQQAFQEPGINVNPILMTKVEEHNKKVRENPRDIQAWMNFVSFQD  342
NRDE2_Cel      321  KIRDRVDLLNLDH-------IDDDVLANDSDNR----KDAFTFELEQAKKTFSENVHNIDALIKVISMEE  379
NRL1_Spo       147  ---RNS--DPSEKSTF---------------------SLNILKAIKETDEEIKKNPGKARLWIKMCEYQE  190
Consensus_aa:       ...c.s....Llp.p.....................p.hh.h.lE-hscplpENs.chphhhphh.hp-
Consensus_ss:                                          hhhhhhhhhhhhhhhhh   hhhhhhhhhhhhh

Conservation:                                      7     55 9 6          7      6
NRDE2_Dme      188  DHWLQL-HRLLNLNL------DKANRLAVAEQQIHSLETAMEH--HPSNQQILRLYTHVANATY-QANEV  247
NRDE2_Hsa      337  EVMKSP-GLYAIEEG--EQEKRKRSLKLILEKKLAILERAIES--NQSSVDLKLAKLKLCTEFW-EPSTL  400
NRDE2_Dre      323  ELAFGS-GSFS-SAGDCDAEMRKMSLRAVLEKKLSIMDRAVES--NPSDVDLKLEKLRLCKELW-EPAVL  387
NRDE2_Mmu      346  EVMRSP-GIYALGEG--EQEKHRKSLKLLLEKKLAVLERAIES--NPGSVELKLAKLQLCSEFW-EPSAL  409
NRDE2_Gga      343  ELMRGP-SPYA-SMG--EQEVRRKSLKLILEKKLAILERAIES--NPSNVDLKLARLSVCTEFW-ESPAV  405
NRDE2_Cel      380  EMCRRNVGSFSSS-----------NPAALAERHQEMVKKAIKA--DGRNAKLRLMKIELLIKMDPNSPTI  436
NRL1_Spo       191  RLLFDE-FRRSNSDD---IKGKLKIENNSRSVKLSILEKALKEVKGCDHEILVSYYLQLGSEEW-SKEET  255
Consensus_aa:       -hh......ht.........c..s..hlhEp+l.hl-+Alcp..ssss.pL..hblpltpch@.pss.l
Consensus_ss:       hhhh               hhhhhhhhhhhhhhhhhhhhhh      hhhhhhhhhhhhh    hhhh

Conservation:             6       9    79 57    7             6  7         6
NRDE2_Dme      248  ARRFEKMLKRNPFQYTLWSNLIMITQGDMACCNVSAVLRIYEYSMRRMHVGHNDE-------ITRRFI--  308
NRDE2_Hsa      401  VKEWQKLIFLHPNNTALWQKYLLFCQSQFSTFSISKIHSLYGKCLSTLSAVKDGS-------ILSHPA--  461
NRDE2_Dre      388  QKEWKKLVFIHPNSAPLWKKYLLFTQSHFSTFSVSKVNGVFGKCLSTLSAVQDGS-------MVSHPP--  448
NRDE2_Mmu      410  AKEWQKLLFLHPNNTSLWQRYLSFCQSQFGTFSVSKLHSLYGKCLSTLSAVKDGS-------MLSHPV--  470
NRDE2_Gga      406  IKEWQKLIFIHPNNPELWKKYLLFCQSQFSTFSVSKINSLYGKCLTTLAAVQDGS-------MVSHPP--  466
NRDE2_Cel      437  IDDFKNLTITFPHEPMVWIKYLDYIQYDSNVYNYKKLKNAFEDCIRQVTGLTNGT-------LLSHLNAV  499
NRL1_Spo       256  NQKFEEVLIEHPGYLNLWMKYAEYFTG-ISEFTFNDCLNMFSKCFKFLKQKLSDRKSCKERESTDVTS--  322
Consensus_aa:       .cc@pcllh.@P.p..LWb+Yl.@hQtphth@shscl.sh@.cChpplps.pssp.......hhs+.s..
Consensus_ss:       hhhhhhhhhh    hhhhhhhhhhhhhhhh   hhhhhhhhhhhhhhhhhh

Conservation:                   7 6    6 77   77       677  6  7                       7 77
NRDE2_Dme      309  --IKETDVIMLKLFHNCLLFLRQSGHTNWMFALIRLSMELNFPYLTFECLQAHAGREKAL--IGFEEVVL  374
NRDE2_Hsa      462  --LPGTEEAMFALFLQQCHFLRQAGHSEKAISLFQAMVDFTFFKPDSVK----DLPTKGQ--VEFFEPFW  523
NRDE2_Dre      449  --LPGTQEDLLDIFLQQCHFLRQAGHTEKAVCLFQALLDFTFFKPDNVK----NLATRQQ--VDFFEPFW  510
NRDE2_Mmu      471  --LPGTEEAMFGLFLQQCHFLRQAGHSEKVISLFQAMVDFTFFKPDSVK----ELPTKVQ--VEFFEPFW  532
NRDE2_Gga      467  --LPGTEEAMLAIFIQQCHFLRQAGHSEKAVSLFQALIDFTFFKPDSVK----DLPTKGQ--VEFFEPFW  528
NRDE2_Cel      500  NDRPLLRMFHLWIYIRYLKWMISCAHTPVVLANIQATFEYNFGLADVEK----RTSTNSKEREVRLEEFW  565
NRL1_Spo       323  --NFEVEEAILHLLIRLCDFLKNCGYYELAWSIFQANMELCYFYPRYLE----KKLDSTF--FESFSKFW  384
Consensus_aa:       .....hcbhhL.l@lpbhcFL+ptGHopbhhtlhQA.h-hsF.bsc..c....p..spsb..h..hE.FW
Consensus_ss:            hhhhhhhhhhhhhhhhh    hhhhhhhhhhhhhh              hhhh  hhhhhh

Conservation:             97 9  66 69696   6              9 6       6 69                    9
NRDE2_Dme      375  RSGMPMP-------------------------------------------------------------EIW  384
NRDE2_Hsa      524  DSGEPRAGEKGARGWKAWMHQQERGGWVVI-NPDEDDDEPEEDDQEIK--------DKTL----PRWQIW  580
NRDE2_Dre      511  DSGEPRIGERGARGWKAWMLQQEKGGWVIPPEPD-DDEEEEQEDSEIK--------DKTW----PKWKIW  567
NRDE2_Mmu      533  DSGEPRVGEKGARGWRAWMHQQERGGWVLI-TPDEDDEEPEEEDQEIK--------DKTL----PRWQIW  589
NRDE2_Gga      529  DSGEPRIGEKGARGWRAWMHQQEKGGWVAVDNPDDDDEEEIDEDEEIK--------DKTL----PKWHIW  586
NRDE2_Cel      566  ESGLPRIGDEGAVGAEKMLKQSE-------ELSDEDIQKLENDDFDILISRTEETIATCLQAQRDVQISW  628
NRL1_Spo       385  NSDTPKFSEENARGWCNVLD------------DESSQQNQNFSSEIGIF--------------QTVKLW  427
Consensus_aa:       cSGbP+ht-csA.Gh..hhc.............D.s.pp.pp.s.-I...................plW
Consensus_ss:                 hhhh                                          h         hhh

Conservation:             9         7                 7 57    7 6                  6   7  6 76
NRDE2_Dme      385  TRVEQLRQAYCFLPLKDKLASSTNVFKNKMNAERYFCSDKLV-PYVH--ALKSQNNRLHLVLVVVQLMKL  451
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NRDE2_Hsa      581  LAAERSRDQRHWRPWRPDKTK-KQTEEDCEDPERQVLFDDIG-QSLI--RLSSHDLQFQLVEAFLQFLGV  646
NRDE2_Dre      568  LDVEASREANQWLPWRPDKTK-GQSEEDCEDPDRQVLFDDIG-PSMI--RVDRADLQAQLILAFLQFLGL  633
NRDE2_Mmu      590  LAVERSRDQRHWRPWRPDKTK-KQTEEDCEDPERQVLFDDIG-QSLI--RLSSPDLQFQLIQAFLQFLGV  655
NRDE2_Gga      587  MDIEYSRESRHWLPWRPDKTK-NQAEEDCEDPERQVLFDDLG-PSLI--QLSNPDIQHQLLYSFLQFLGV  652
NRDE2_Cel      629  IEVEREMMNIDARVKRTKLKDCELYEDHVDDLETCELWDIIPFDRIRYYEAPGDCANFDFVQPFLELLGV  698
NRL1_Spo       428  YLNESKFDT-NPPPRSTMSCR---KLSGIDDPFRYIVFNDIQ-DFIV--CFESETIAFAFKYKFFAFCGV  490
Consensus aa:       h.hE.p.ps.ph.Pb+scbhc.p..bcsh-Ds-Rbhl@Dcls.s.lh...hps.shphphlbsFlphhGl
Consensus ss:       hhhhhhhhhh                    eeeeee    hhhh  e   hhhhhhhhhhhhhh

Conservation:       7                 6                 6
NRDE2_Dme      452  P-LIR--SASLADKLNPCIEDFGESEAIEMLF-----AAIVDRHSYA---VASLQYQFDA-AMIDLAREM  509
NRDE2_Hsa      647  P---S--GFTPPASCLYLAMDE--------------NSIFDNGLYDEKPLTFFNPLFSGASCVGRMDRL  696
NRDE2_Dre      634  PGALT--KFFTASSSSFL-LDD--------------LTFLEEGPDPERPLTSYDLPVTGICAVGHMTCL  685
NRDE2_Mmu      656  P---S--GFLPPASCLYLAMDE--------------SSIFESELYDEKPLTYFNPSFSGISCVGSMEQL  705
NRDE2_Gga      653  P-CIS--RLFPPN--LYIAMDE--------------NNIFDNVLSDEKPLTSIDTPLSGFNSIGHMDTM  702
NRDE2_Cel      699  K-FLN--STNCFTTT--------------------EQIISDWISNDSTVNFYKTP-TYT---------  733
NRL1_Spo       491  PLFPPGISTNSW------FAS-YDKGIYNLLFGMASSESFINGQIAEKNSFQFP--------------C  538
Consensus aa:       P...s..ph.s.s.......s.................shhpp...scpsls.hp...s.........h
Consensus ss:                       hhhh         hhhh

Conservation:                                                 65         5    7 7
NRDE2_Dme      510  SVTPSYMPHLVGHKLYADT-----------VSNMLLKCVE--VFIGEESKRRLLLILYLRFQKLLIV--  563
NRDE2_Hsa      697  GYPRWTRGQNRE---GEEF-----------IRNVFHLVMP--LFSGKE--KSQLCFSWLQYEIAKVIWC  747
NRDE2_Dre      686  SGCRRQAGLCKA---GEEF-----------LHNVLHQTLR--LVPAQD--QAAITLCWLQYEKLKVLRC  736
NRDE2_Mmu      706  GRPRWTKGHNRE---GEEF-----------VRNVFHLVLP--LLAGKQ--KSQLSLSWLRYEIAKVIWC  756
NRDE2_Gga      703  LRRRHHIGHCKD---GEEF-----------IQNVFHVLSP--LFSGKE--KSNLSICWLQYEISKVVQC  753
NRDE2_Cel      734  --EKKCFEVGNN---ILKF-----------MLYNRLKL------TENN--PEYLDKTMVKYLLAMLVTE  778
NRL1_Spo       539  SILPSYIDLFISLMSFKNLNFKLFDYNLAHHVKESMERAFHQLVFSADDEYLASV---YLIYLKQMETKN  605
Consensus aa:       t...phhsh..s....bch...........lppshh.hh...lhstpp.....l.b.@LpYbb.bll..
Consensus ss:          hhhhhh     hhhhh            hhhhhhhhhh  h  hhhhhhhhhhhhhhhhhhhhhh

Conservation:       5    67      7   5        6 7 66   5         7  9        7
NRDE2_Dme      564  LHKSMGK----LTKKFFKDKRTRMRKLINLTE--NRNVISFYTEQALCEIEAKHYRLGFSIISRIITKKS  627
NRDE2_Hsa      748  LHTKNKK----RLKSQGKNCKKLAKNLLKEPE--NCNNFCLWKQYAHLEWLLGNTEDARKVFDTALGMAG  811
NRDE2_Dre      737  LRSGNKR----QMKSQGKRSKRLAKRLLKEPE--NRGSLALWREYAHLEWLLGNLEEARKVFDTALSMGV  800
NRDE2_Mmu      757  LHTK-KK----RLKSQGKSCKKLAKNLLKEPE--NRNNFCLWKQYAHLEWLLGNTEDARKVFDTALSMAG  819
NRDE2_Gga      754  IQTKKKK----KLKAQGRKSKKLAKNLLKAPD--NRNNLALWKLYAYLEWLLGNTDDARKVFDTALCTAG  817
NRDE2_Cel      779  ASEQEKKLNFHSFKLNLKNL--VGTFITKHPDIFKRAMLSKITGIVYMEKFVSWWERALKEQEKVVEADE  846
NRL1_Spo       606  L------------SEEKPQVNKIVKKILKK----YDSSVSVWNTYAQLEHLSGAFTMAETIFKTIFQIHA  659
Consensus aa:       lppp..K.....hK.p.+p.pplh+pllK.s-..pcsslth@p.hAhhEhhht.hc.A.plhcphl.h.s
Consensus ss:       h hhhhh    hhhhhhhhhhhhhhhhhh         hhhhhhhhhhhhh   hhhhhhhhhhhhhhh

Conservation:                       7
NRDE2_Dme      628  EESSCISCVDTMHLYLVCAEL--------------------------------HIHRGRI-------SKA  658
NRDE2_Hsa      812  --SRELKDSDLCELSLLYAEL--------------------------------EVELSPEVRRAAT-ARA  846
NRDE2_Dre      801  --SRGLNDHVLCKLCFLYAQL--------------------------------EVEQALSSETVSTFSKA  836
NRDE2_Mmu      820  --SSELKDRELCELSLLYAEL--------------------------------EMELSPDSRGATT-GRA  854
NRDE2_Gga      818  --TGGLKSPQLCSLSLLYAQL--------------------------------EVELLQSLEGAVT-SRA  852
NRDE2_Cel      847  --RRKNYK-EIKMEEGVVDDVKFDVILLKKDKERVQTIRDKIRDMIDIAIPKSTEKLIQSADSSLP-TLQ  912
NRL1_Spo       660  ---SQLRYIDNLNVYKNWAFR--------------------------------KLLINDTEGC------L  688
Consensus aa:       ..pp.lp..phhpl.blhApl.....................................chcb..p..s....s.h
Consensus ss:          h    hhhhhhhhhhhhh                                    hhhhh         hhh

Conservation:         7   7  6                6
NRDE2_Dme      659  IGILVSLCLEKHAGVKQTTVYSKEQVLKGLRNAEKMVRAELKSLDTQPAEMSLEEYFQ------------  716
NRDE2_Hsa      847  VHILTKLT-ESSPYGPYTGQVLAVHILKARKAYEHALQDCLGDSCVS----NPAPTDS------------  899
NRDE2_Dre      837  VYILTKLA-EGASYTPFSGQINPVVILKARKAYEQALLSILPEQNTD----SNAGASK------------  889
NRDE2_Mmu      855  VHILTRLT-ESSPYGPYTGQVSSTQVLKARKAYELALQDCLGQSCAS----SPAPAEA------------  907
NRDE2_Gga      853  VHILTKLA-ESGPYAPYNGQVLSVSVLKARKTYEHALQDYLNKAPVS----DQDGASN------------  905
NRDE2_Cel      913  LHLYANVL--------------RGRLSILNQNALEETRDVFCKEILG----IHTSEFESDEALLLALDQG  964
NRL1_Spo       689  VIIKCLLF-PGDKSLTSDNN-------RASEMLFGMLENCASK--------------------------  723
Consensus aa:       lhIhhpLh.........ss.....pl.+tpcshb.hlpshhsp..h...........p.....
Consensus ss:       hhhhhhhh hh        h  hhhhhhhhhhhhhhhhhhhhhh           hhh
```

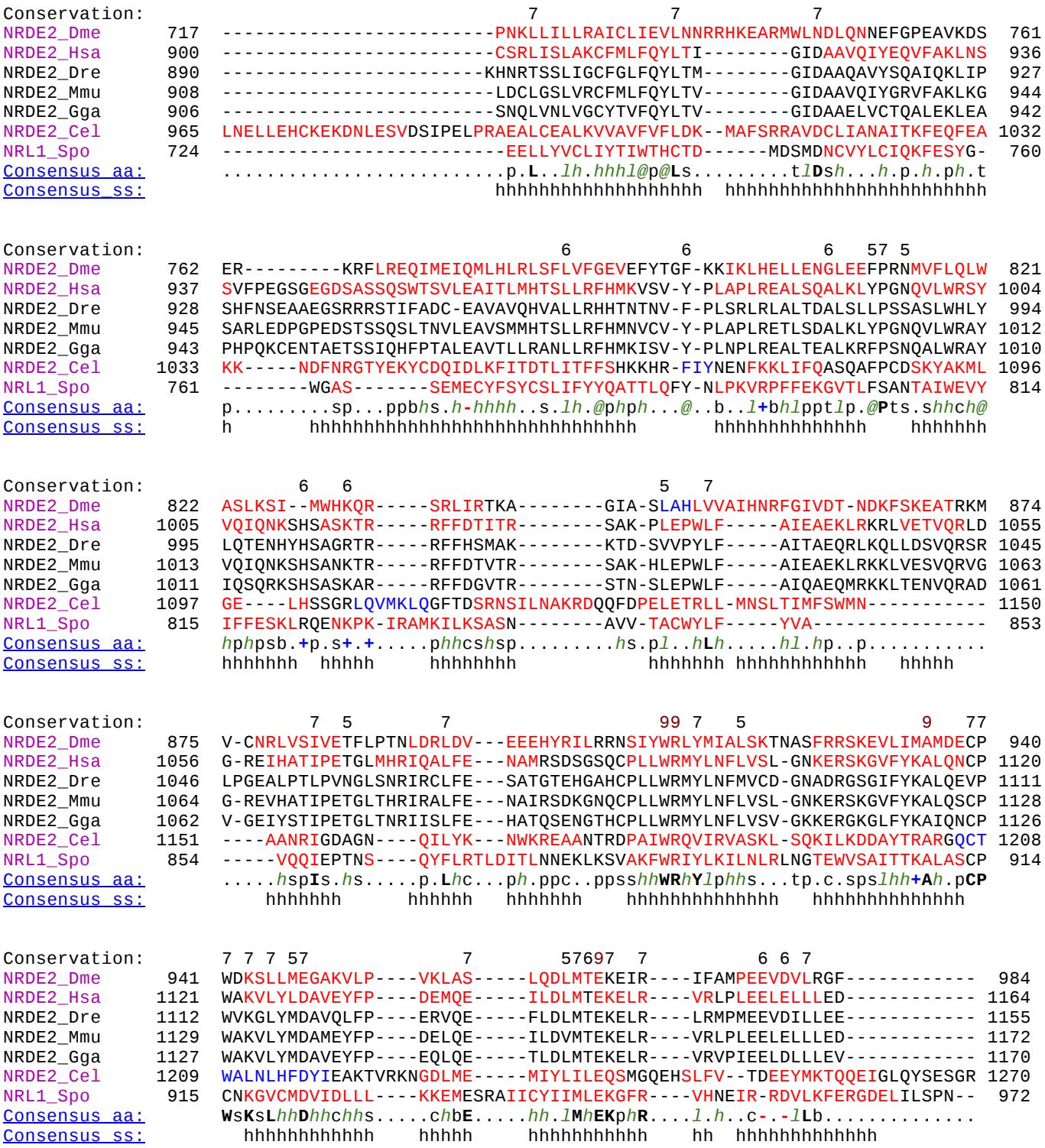

Figure S1. NRDE-2 like proteins are conserved in many eukayotes. NRDE-2 like proteins from *S. pombe* (Spo), *C. elegans* (Cele), *Danio rerio* (Dre), *D. melanogaster* (Dme), *Mus musculus* (Mmu), *G. gallus* (Gga) and *H. sapiens* (Hsa), were aligned using PROMALS3D to obtain secondary structure, conservation, and consensus sequence information. Aminoacid sequences are colored according to their predicted secondary structures (red: alpha-helix, blue: beta-strand). The first line in each block shows conservation indices for positions with a conservation index above 4. The last two lines show consensus amino acid sequence (Consensus_aa) and consensus predicted secondary structures (Consensus_ss). Consensus predicted secondary structure symbols: alpha-helix: h; beta-strand: e. Conserved amino acids are in bold and uppercase letters; aliphatic (I, V, L): l; aromatic (Y, H, W, F): @; hydrophobic (W, F, Y, M, L, I, V, A, C, T, H): h; alcohol (S, T): o; polar residues (D, E, H, K, N, Q, R, S, T): p; tiny (A, G, C, S): t; small (A, G, C, S, V, N, D, T, P): s; bulky residues (E, F, I, K, L, M, Q, R, W, Y): b; positively charged (K, R, H): +; negatively charged (D, E): -; charged (D, E, K, R, H): c.

Colored PROMALS3D alignment (sequences in aligned order)

```
Conservation:          9
CWF4_Spo           1  MTS-------------------------------------------------------------------    3
SYF3_human         1  MTA-----------------------------------TVEN-----LTFQKDT------LGNAVDKN-   22
CWF3_Spo           1  M-------------------------------------------------------------------    1
SYF1_human         1  MV------------------------------------------------------------------    2
NRDE2_Cele         1  MF--RAYGNNGLKNPERISGENPDLYTQTRAAVQQRATTTLKRNEKQKLAVQNDSVFQQVGIGESDSDDD   68
NRDE2_human        1  MALFPAFA--------------------------------------------------GLSEAPDGG-   17
NRL1_Spo           1  MPS-------------------------------------------------------------NH    5
Consensus aa:          Mh.....................................................................
Consensus ss:

Conservation:
CWF4_Spo               ------------------------------------------------------------------
SYF3_human        23   --TSRLELR----------------------SYSLAGRH---------------GSTEPLVLAWSSQF   51
CWF3_Spo               ------------------------------------------------------------------
SYF1_human             ------------------------------------------------------------------
NRDE2_Cele        69   NGGVRIRMSPHRYIDPDDVFTLPEVKKQNALRDAKIAARAAQATAYNTFPSVKSLNGCQDPPETSQQSTS  138
NRDE2_human       18   --SSRKEL---------DWLSNPSFCVGSIT-------------------------------SLSQQTEA   45
NRL1_Spo           6   N-TSVPKF-------------------------------------SSFNSVKA----------------   20
Consensus aa:          .....................................................................
Consensus ss:

Conservation:
CWF4_Spo               ------------------------------------------------------------------
SYF3_human        52   RRLTWGCALDALHRSPCVAA-SQHGVTH--LIRS----------------------------SRTPHS-   88
CWF3_Spo               ------------------------------------------------------------------
SYF1_human             ------------------------------------------------------------------
NRDE2_Cele       139   RKRSASNSR-SPSRSHSRRYDRD----NGRQRSRSREKKRRKKERRR-----------KRSSSRSSS--  189
NRDE2_human       46   APAHVSEGL-PLTRSHLKSESSDESDTNKKLKQTSRKKKKEKKKKRKHQHHKKTKRKHGPSSSSRSETDT  114
NRL1_Spo          21   ------KKN-PITKSN-----------------------------------KKYRSSHDQVSSNHA----   44
Consensus aa:          .....................................................................
Consensus ss:

Conservation:
CWF4_Spo               ------------------------------------------------------------------
SYF3_human        89   -------TRCRKEDAQPGHHGNGAASVTAQAR--GQR-------------SVLQVPL----------PV  125
CWF3_Spo               ------------------------------------------------------------------
SYF1_human             ------------------------------------------------------------------
NRDE2_Cele       190   ------SSRSRDRSSRARDTSSHTLMKMNKPA-KYAFLTDEEYRTCDAYISSAFITQTKSDCENYTQG-V  251
NRDE2_human      115   DSEKDKPSRGVGGSKKESEEPNQGNNAAADTGHRFVWLED-----IQAVTGETFRTDKKPDPANWEYKSL  179
NRL1_Spo          45   ---------------------------------KSSFPSH-------RSIQSNFAVDTKGEKQNLLYG-I   73
Consensus aa:          .....................................................................
Consensus ss:

Conservation:
CWF4_Spo           4   ---------------------------------------------------EAPRVKNKNPAPIQIS   19
SYF3_human       126   PRS----CLFSESFVVSVSSQ-SRFLASVPGTGVQRSTAADMAASTAAGKQRIPKVAKVKNKAPAEVQIT  190
CWF3_Spo           2   ---------------------------------------------------GDVIPLKVNFDLIN   15
SYF1_human         3   ---------------------------------------------------VMARLSRPERPDLVFE   18
NRDE2_Cele       252   PKKEIAKCRLSVKFIVGLEHN-NILFNNIYGA---------------------EYARDKENRP-----F  293
NRDE2_human      180   YRGDIARYKRKGDSCLGINPK-KQCISWEGTS---------------------TEKKHSRK--------  218
NRL1_Spo          74   NKRPVPKYHRSSSSVYGSAPLLRIVKESKEGI---------------------TLNKKK---SLEIKYD  118
Consensus aa:          ...................................................phs+.pp..s......
Consensus ss:                      eee                                   h             h

Conservation:            65 5
CWF4_Spo          20   AEQLLREAVERQ-------------------DVA-------------------FVPP-KINITDLEEL--   48
SYF3_human       191   AEQLLREAKEREL-----------------ELL-------------------PPPP-QQKITDEEEL--  220
CWF3_Spo          16   VDDEPFELELLR-------------------DPY-------------------SLKS-WLRYIKTHE---   43
SYF1_human        19   EEDLPYEEEIMR-------------------NQF-------------------SVKC-WLRYIEFKQ---   46
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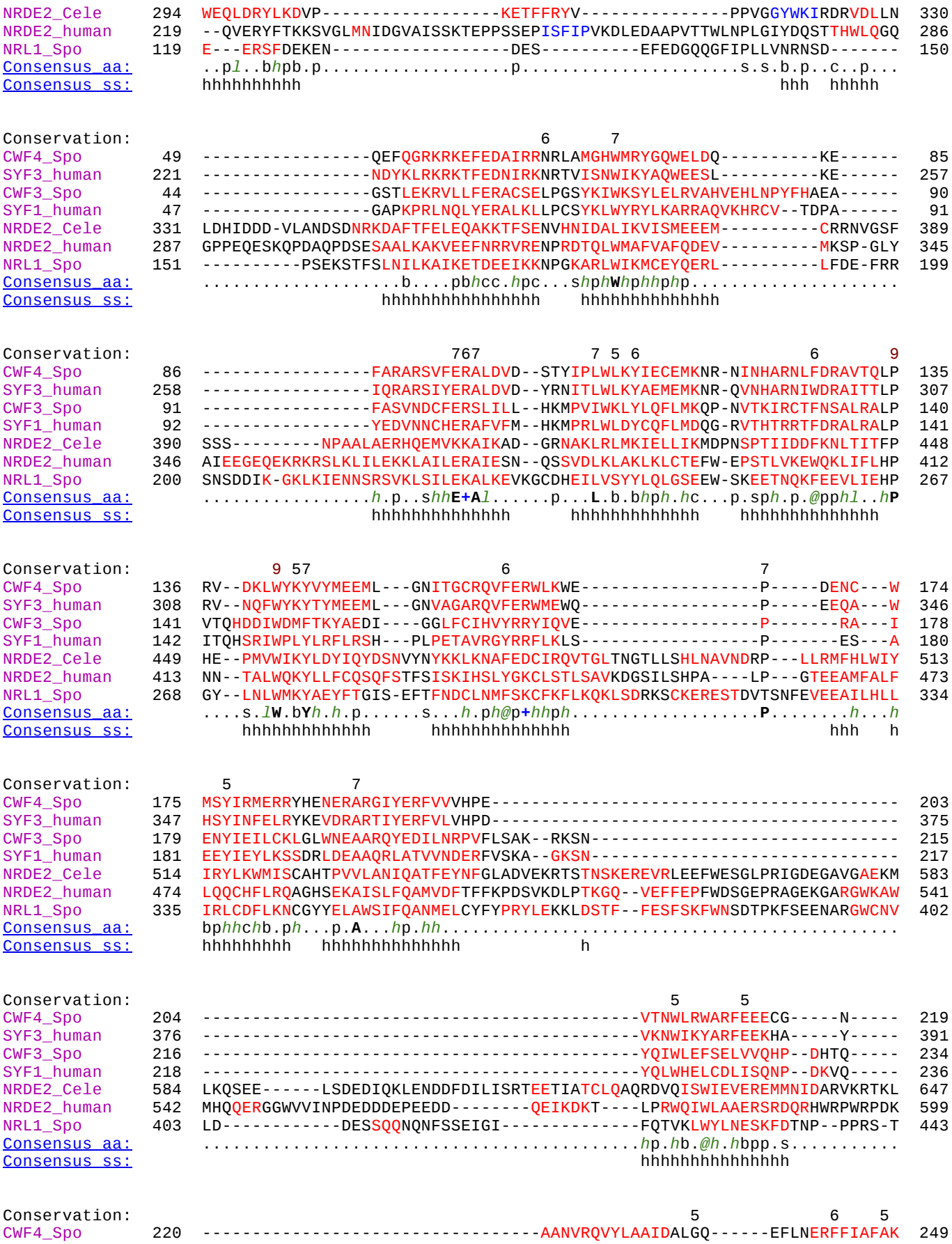
NRDE2_Cele 294 WEQLDRYLKDVP----------KETFFRYV----------PPVGGYWKIRDRVDLLN 330
NRDE2_human 219 --QVERYFTKKSVGLMNIDGVAISSKTEPPSSEPISFIPVKDLEDAAPVTTWLNPLGIYDQSTTHWLQGQ 286
NRL1_Spo 119 E---ERSFDEKEN----------DES----------EFEDGQQGFIPLLVNRNSD------- 150
Consensus_aa:
Consensus ss:
Conservation:
CWF4_Spo 49 ----------QEFQGRKRKEFEDAIRRNRLAMGHWMRYGQWELDQ----------KE------ 85
SYF3_human 221 ----------NDYKLRKRKTFEDNIRKNRTVISNWIKYAQWEESL----------KE------ 257
CWF3_Spo 44 ----------GSTLEKRVLLFERACSELPGSYKIWKSYLELRVAHVEHLNPYFHAEA------ 90
SYF1_human 47 ----------GAPKPRLNQLYERALKLLPCSYKLWYRYLKARRAQVKHRCV--TDPA------ 91
NRDE2_Cele 331 LDHIDDD-VLANDSDNRKDAFTFELEQAKKTFSENVHNIDALIKVISMEEEM----------CRRNVGSF 389
NRDE2_human 287 GPPEQESKQPDAQPDSESAALKAKVEEFNRRVRENPRDTQLWMAFVAFQDEV----------MKSP-GLY 345
NRL1_Spo 151 ----------PSEKSTFSLNILKAIKETDEEIKKNPGKARLWIKMCEYQERL----------LFDE-FRR 199
Consensus_aa:
Consensus ss:
Conservation:
CWF4_Spo 86 ----------FARARSVFERALDVD--STYIPLWLKYIECEMKNR-NINHARNLFDRAVTQLP 135
SYF3_human 258 ----------IQRARSIYERALDVD--YRNITLWLKYAEMEMKNR-QVNHARNIWDRAITTLP 307
CWF3_Spo 91 ----------FASVNDCFERSLILL--HKMPVIWKLYLQFLMKQP-NVTKIRCTFNSALRALP 140
SYF1_human 92 ----------YEDVNNCHERAFVFM--HKMPRLWLDYCQFLMDQG-RVTHTRRTFDRALRALP 141
NRDE2_Cele 390 SSS---------NPAALAERHQEMVKKAIKAD--GRNAKLRLMKIELLIKMDPNSPTIIDDFKNLTITFP 448
NRDE2_human 346 AIEEGEQEKRKRSLKLILEKKLAILERAIESN--QSSVDLKLAKLKLCTEFW-EPSTLVKEWQKLIFLHP 412
NRL1_Spo 200 SNSDDIK-GKLKIENNSRSVKLSILEKALKEVKGCDHEILVSYYLQLGSEEW-SKEETNQKFEEVLIEHP 267
Consensus_aa:
Consensus ss:
Conservation:
CWF4_Spo 136 RV--DKLWYKYVYMEEML---GNITGCRQVFERWLKWE----------P-----DENC---W 174
SYF3_human 308 RV--NQFWYKYTYMEEML---GNVAGARQVFERWMEWQ----------P-----EEQA---W 346
CWF3_Spo 141 VTQHDDIWDMFTKYAEDI----GGLFCIHVYRRYIQVE----------P-------RA---I 178
SYF1_human 142 ITQHSRIWPLYLRFLRSH---PLPETAVRGYRRFLKLS----------P-------ES---A 180
NRDE2_Cele 449 HE--PMVWIKYLDYIQYDSNVYNYKKLKNAFEDCIRQVTGLTNGTLLSHLNAVNDRP---LLRMFHLWIY 513
NRDE2_human 413 NN--TALWQKYLLFCQSQFSTFSISKIHSLYGKCLSTLSAVKDGSILSHPA----LP---GTEEAMFALF 473
NRL1_Spo 268 GY--LNLWMKYAEYFTGIS-EFTFNDCLNMFSKCFKFLKQKLSDRKSCKEREST----DVTSNFEVEEAILHLL 334
Consensus_aa:
Consensus ss:
Conservation:
CWF4_Spo 175 MSYIRMERRYHENERARGIYERFVVVHPE---------- 203
SYF3_human 347 HSYINFELRYKEVDRARTIYERFVLVHPD---------- 375
CWF3_Spo 179 ENYIEILCKLGLWNEAARQYEDILNRPVFLSAK--RKSN---------- 215
SYF1_human 181 EEYIEYLKSSDRLDEAAQRLATVVNDERFVSKA--GKSN---------- 217
NRDE2_Cele 514 IRYLKWMISCAHTPVVLANIQATFEYNFGLADVEKRTSTNSKEREVRLEEFWESGLPRIGDEGAVGAEKM 583
NRDE2_human 474 LQQCHFLRQAGHSEKAISLFQAMVDFTFFKPDSVKDLPTKGQ--VEFFEPFWDSGEPRAGEKGARGWKAW 541
NRL1_Spo 335 IRLCDFLKNCGYYELAWSIFQANMELCYFYPRYLEKKLDSTF--FESFSKFWNSDTPKFSEENARGWCNV 402
Consensus_aa:
Consensus ss:
Conservation:
CWF4_Spo 204 ----------VTNWLRWARFEEECG-----N----- 219
SYF3_human 376 ----------VKNWIKYARFEEKHA-----Y----- 391
CWF3_Spo 216 ----------YQIWLEFSELVVQHP--DHTQ----- 234
SYF1_human 218 ----------YQLWHELCDLISQNP--DKVQ----- 236
NRDE2_Cele 584 LKQSEE------LSDEDIQKLENDDFDILISRTEETIATCLQAQRDVQISWIEVEREMMNIDARVKRTKL 647
NRDE2_human 542 MHQQERGGWVVINPDEDDDEPEEDD--------QEIKDKT----LPRWQIWLAAERSRDQRHWRPWRPDK 599
NRL1_Spo 403 LD----------DESSQQNQNFSSEIGI----------FQTVKLWYLNESKFDTNP--PPRS-T 443
Consensus_aa:
Consensus ss:
Conservation:
CWF4_Spo 220 ----------AANVRQVYLAAIDALGQ------EFLNERFFIAFAK 249

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SYF3_human     392  ---------------------------------FAHARKVYERAVEFFGD------EHMDEHLYVAFAK  421
CWF3_Spo       235  ---------------------------------NIDVEKVFRAGIKRFSD------Q--AGKLWTYLAQ  262
SYF1_human     237  ---------------------------------SLNVDAIIRGGLTRFTD------Q--LGKLWCSLAD  264
NRDE2_Cele     648  KDCELYEDHVDDLETCELWDIIPFDRIRYYEAPGDCANFDFVQPFLELLGVKFLN--S--TNCFTTTEQ-  712
NRDE2_human    600  TKKQ-TEEDCEDPERQVLFDDIG-QSLIRLS--SHDLQFQLVEAFLQFLGVPSGFTPP--ASCLYLAMDE  663
NRL1_Spo       444  MSCR-KLSGIDDPFRYIVFNDIQ-DFIVCFE--SETIAFAFKYKFFAFCGVPLFPPGIS-TNS-------  501
Consensus aa:       .....................................h.s..hh...lp.hts.........s..hhhhb..
Consensus ss:                                          hhhhhhhhhhhhhh             hhhhhhhhh


Conservation:         5
CWF4_Spo       250  FEIRQK------------------------------------------------------------------  255
SYF3_human     422  FEENQK------------------------------------------------------------------  427
CWF3_Spo       263  YYIRIG------------------------------------------------------------------  268
SYF1_human     265  YYIRSG------------------------------------------------------------------  270
NRDE2_Cele     713  --------------------IISDWISN---------DSTVNFYKT--PTYTEKKC-------------  737
NRDE2_human    664  NSIFDN--------------GLYDEKPLTFFNPLFSGASCVGRMDR--LGYP-RWT-------------  702
NRL1_Spo       502  WFASYDKGIYNLLFGMASSESFINGQIAE---------KNSFQFPCSILPSYIDLFISLMSFKNLNFKLF  562
Consensus aa:       @bhp..................................................................
Consensus ss:       hhhh


Conservation:                5                                7
CWF4_Spo       256  ------EYERARTIFKYAIDF---MPRS-KSMELYKEYTHFEKQFGDH----------------------  293
SYF3_human     428  ------EFERVRVIYKYALDR---ISKQ-DAQELFKNYTIFEKKFGDR----------------------  465
CWF3_Spo       269  ------DYEKARSTFYEGMNN---IMTVRNFTIIFDAFVEFEEQWLSA----------------------  307
SYF1_human     271  ------HFEKARDVYEEAIRT---VMTVRDFTQVFDSYAQFEESMIAA----------------------  309
NRDE2_Cele     738  -FEVGNNILKFMLYNRLKLTENNPEYLDKTMVKYLLAMLVTEASEQEKKLNFHSFKLNLKNLVGTFITKH  806
NRDE2_human    703  -RGQNREGEEFIRNVFHLVMP---LFSGKEKSQLCFSWLQYEIAKVIW----------------------  746
NRL1_Spo       563  DYNLAHHVKESMERAFHQL-----VFSADD-EYLASVYLIYLKQMETK----------------------  604
Consensus aa:       ......chcch...hb..lp....h.ps.p..blh.s@h.@Ebpb.....................
Consensus ss:              hhhhhhhhhhhhhh       hhhhhhhhhhhhhhhhhhhh


Conservation:
CWF4_Spo       294  --------LGVESTV-------------------------LDKRRL-QYEKLLKDSPYDYDTWLDLLKL  328
SYF3_human     466  --------RGIEDII-------------------------VSKRRF-QYEEEVKANPHNYDAWFDYLRL  500
CWF3_Spo       308  --------RVEASSGNA---NDELSIDFHMAWLEKI-----LDKRPLYINDVLLRQNINNVDEWLRRVKF  361
SYF1_human     310  --------KMETASELGREEEDDVDLELRLARFEQL-----ISRRPLLLNSVLLRQNPHHVHEWHKRVAL  366
NRDE2_Cele     807  PDIFKRAMLSKITGI--------------VYMEKFVSWWERALKEQEK-VVEADERRKNY---------  851
NRDE2_human    747  --------CLHTKNK--------------KRLKSQ----GKNCKKLAKNLLKEPENCNNF---------  780
NRL1_Spo       605  --------NLSEE---------------------------KPQVNKIVKKILK--KYDSS----------  627
Consensus aa:       ............ps.........................s.+.h.b...bhcpp.psh.........
Consensus ss:              hhhhhhh               hhhhhh     hhhhhhhhhhhhhh     hhhhhhhhhh


Conservation:                                          5   5             5 6
CWF4_Spo       329  EESAGDINTIRETYEKAIAKVPEVVEKNAWRRYVYIWLNYCLFEEIDVKDVDRARKVYQEALKLIPH---  395
SYF3_human     501  VESDAEAEAVREVYERAIANVPPIQEKRHWKRYIYLWINYALYEELEAKDPERTRQVYQASLELIPH---  567
CWF3_Spo       362  LEDDS--EKVVQVYTDAIKNVNPK---LAHGSLGKLFSEFARFYE-NFDDLEQSRIIFEKATHVPYK---  422
SYF1_human     367  HQGRP--REIINTYTEAVQTVDPF---KATGKPHTLWVAFAKFYE-DNGQLDDARVILEKATKVNFK---  427
NRDE2_Cele     852  ---------------------------------KEIKMEEGVVDD-------VKFDVILLKKDKERVQTI  881
NRDE2_human    781  ---------------------------------CLWKQYAHLEW-LLGNTEDARKVFDTALGMAGSREL  815
NRL1_Spo       628  ---------------------------------VSVWNTYAQLEH-LSGAFTMAETIFKTIFQIHASQLR  663
Consensus aa:       ................................l@.p@t.hbc....sh-.sc.lhp..hph..p...
Consensus ss:       h       hhhhhhhhhhhhh             hhhhhhhhhhhh h   hhhhhhhhhhhhhh


Conservation:           5      5  5              5
CWF4_Spo       396  KKFTFAKLWLMYAMFELRQR------KIDVARKTLGRALGMCP------------------KPKLFRGY  440
SYF3_human     568  KKFTFAKMWILYAQFEIRQK------NLSLARRALGTSIGKCP------------------KNKLFKVY  612
CWF3_Spo       423  TVNELAQVWIDWAEMELRHQ------NFDAARKLIGDAVHAPRKSHISFFDESLSPQVRLHKSSKIWMYY  486
SYF1_human     428  QVDDLASVWCQCGELELRHE------NYDEALRLLRKATALPARR-AEYFDGSEPVQNRVYKSLKVWSML  490
NRDE2_Cele     882  RDKIRDMIDIAIPKSTEKLIQSADSSLPTLQLHLYANV---------LRG------------RLSILNQN  930
NRDE2_human    816  KDSDLCELSLLYAELEVELSPEVRRAATARAVHILTKLTESSPY---GPY------------TGQVLAVH  870
NRL1_Spo       664  YIDNL-NVYKNWAFRKLLIN------DTEGCLVIIKCLLFPGDKS--LTS-----------DNNRASEML  713
Consensus aa:       p..phspl.h.htb.clc.p.......hs.t.+hl.phh..ss..................p.plh..h
Consensus ss:        hhhhhhhhhhhhhhhhh         hhhhhhhhhhhhhh                    hhhhhhhh
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Conservation:          5                5         7
CWF4_Spo        441  IEFEDAIK----------QFDRC---RILYEKWILYDP----------------------------E  466
SYF3_human      613  IELELQLR----------EFDRC---RKLYEKFLEFGP----------------------------E  638
CWF3_Spo        487  LDLEESVG----------TIETT---RKLYDRVFELKI----------------------------A  512
SYF1_human      491  ADLEESLG----------TFQST---KAVYDRILDLRI----------------------------A  516
NRDE2_Cele      931  ALEETRDVFCKEILGIHTSEFESD---EALLLALDQGLN----------------------------E  967
NRDE2_human     871  ILKA----------------------RKAYE------------------------------------H  880
NRL1_Spo        714  FGMLENCA----------SKEELLYVCLIYTIWTHCTDMDSMDNCVYLCIQKFESYGWGASSEMECYFS  772
Consensus aa:          h.bbpp.............pb-p....c.lYp.h.p.................................
Consensus ss:          hhhhhh              hhhh   hhhhhhhhhh

Conservation:                                        5                     55     5
CWF4_Spo        467  ACAP------------------------WLGYAALETKLGD------SDRARALYNLAVNQPI------  499
SYF3_human      639  NCTS------------------------WIKFAELETILGD------IDRARAIYELAISQPR------  671
CWF3_Spo        513  TPQV------------------------VVNYANLLEENAY------FEDSFKIYERGVALFS------  545
SYF1_human      517  TPQI------------------------VINYAMFLEEHKY------FEESFKAYERGISLFK------  549
NRDE2_Cele      968  LLEHCKEKDNLESVDSIPELPRAEALCEALKVVAVFVFLDKMAFSRRAVDCLIANAITKFEQFEAK----1033
NRDE2_human     881  ALQDCLGDSC---VSNPAPTDSCSRLISLAKCFMLFQYLTIG------IDAAVQIYEQVFAKLNSSVFPE  941
NRL1_Spo        773  YCSL------------------------IFYYQATTLQFYN------LPKVRPFFEKGVTLFS------  805
Consensus aa:          h.p.........................hb.@h.h..b.........h-.s..h@p.shsb.p......
Consensus ss:           hhh                        hhhhhhhhhh         hhhhhhhhhhhhhh

Conservation:                          5  5                     5    566   5       6  66
CWF4_Spo        500  ------------LETPELVWKAYIDFEFEEM------EYG-KARSIYQQLLRTAPH---VKVWISFANFE  547
SYF3_human      672  ------------LDMPEVLWKSYIDFEIEQE------ETE-RTRNLYRRLLQRTQH---VKVWISFAQFE  719
CWF3_Spo        546  ------------YPVAFELWNLYLTKFVKRYQGT---HME-RTRDLFEQALEGCPPEFSKSIYLLYADFE  599
SYF1_human      550  ------------WPNVSDIWSTYLTKFIARYGGR---KLE-RARDLFEQALDGCPPKYAKTLYLLYAQLE  603
NRDE2_Cele     1034  -KNDFNRGTYEKYCDQIDLKFITDTLITFFSHKKHRFIYNENFKKLIFQASQAFPCD--SKY----AKML 1096
NRDE2_human     942  GSGEGDSASSQSWTSVLEAITLMHTSLLRFHMKVSVYPLA-PLREALSQALKLYPGN--QVLWRSYVQIQ 1008
NRL1_Spo        806  ------------ANT--AIWEVYIFFESKLRQEN--KPKI-RAMKILKSASNAVV---TACWYLFYVAVQ  855
Consensus aa:          ............hss.b.lhphhhsbbh..........h..ph+plhpph.p.hs......h@l.@hphp
Consensus ss:                     hhhhhhhhhhhhhhhh       hh hhhhhhhhhhh      hhhhhhhhhhh

Conservation:                                                    5     5
CWF4_Spo        548  I-AHLE-----------------DDDEEPPN----EEV-ASPTAVVRARNVFENAL-AHLRQQGLKEERV  593
SYF3_human      720  L-----------------------------------SSGKEGSLTKCRQIYEEAN-KTMRNCEEKEERL  752
CWF3_Spo        600  E-KFGKAKRSISI-LEKAADKVKTADRLAIYNVLLVKV-ALNYGVLATRTVYEKAI-ESLSDSEVKDMCL  665
SYF1_human      604  E-EWGLARHAMAV-YERATRAVEPAQQYDMFNIYIKRA-AEIYGVTHTRGIYQKAI-EVLSDEHAREMCL  669
NRDE2_Cele     1097  G-ELHSSGRLQVMKLQGFTDSRNSI-LNAKRDQQ--------FDPELETRLLMNSLTIMFSWMNA-----1151
NRDE2_human    1009  N-KSHSASKTRRF-FDTITRSAKPL-EPWLFAIE----------AEKLRKRLVETV-QRLDGREI-----1059
NRL1_Spo        856  QIEPTNSQYFLRT-LDITLNNEKLKSVAKFWRIYLKILNLRLNGTEWVSAITTKAL-ASCPCN--KGVCM  921
Consensus aa:          ..c..p.................s.....b.............sh..hp.lh.ptl...hp..ph.....
Consensus ss:          h h   hhhhhhh hhhhh h      hhhhhhh hhh hh   hhhhhhhhhhhh hh    hhhhhhh

Conservation:                                            5  5        5
CWF4_Spo        594  V---------------------------------LLEAWKQFEAM----HGTE-DTRKHVSSLMPQVVKKR  626
SYF3_human      753  M---------------------------------LLESWRSFEEE----FGTA-SDKERVDKLMPEKVKKR  785
CWF3_Spo        666  RFAEMETKLGEIDRARLIYIHGSQYCDPRVETDYWKAWQEFEIR----YGNPEETVKEM-------LRIK  724
SYF1_human      670  RFADMECKLGEIDRARAIYSFCSQICDPRTTGAFWQTWKDFEVR----HGNE-DTIKEM-------LRIR  727
NRDE2_Cele     1152  ---------------------ANRIGDAG-NQILYKNWKREAAN----TRDP-AIWRQV-------IRVA 1187
NRDE2_human    1060  ---------------------HATIPETG-LMHRIQALFENAMRSDSGSQCP-LLWRMY-------LNFL 1099
NRL1_Spo        922  DVIDLLLKK--EMESRAIICYIIM------------LEKGFRVH----N----EIRRDV-------LKFE  962
Consensus aa:          ..................................hhpshppb.hp......s...h.+ph.......l+b.
Consensus ss:          h                     hhhh      hhhhhhhhhhhhh       h hhhhhh       hhhh

Conservation:          5                              6                          5  5
CWF4_Spo        627  RRLED-----GSFEEY-----------LDYLFPDTATDQGDKM-------RKMLELSRKWKEEMAKKKLE  673
SYF3_human      786  RKVQTDDGSDAGWEEY-----------FDYIFPEDAANQPN---------LKLLAMAKLWKKQQQEKEDA  835
CWF3_Spo        725  RSVQTKFSTDSLHI--------------AKRAAKIESAAA-----------PMDPMEQLEMEKSEGPKAL  769
SYF1_human      728  RSVQATYNTQVNFM--------------ASQMLKVSGSATGTVSDLAPGQSGMDDMKLLEQRAEQLAAEA  783
NRDE2_Cele     1188  SKLSQKILKDDAYTRARGQCTWALNLHFDYIEAKT--VRK-----------NGDLMEMIYLILEQSMG--1242
NRDE2_human    1100  VSLGNKERSKGVFYKALQNCPWAKVLYLDAVEYFP-----------------DEMQEILDLMTEKE----1148
NRL1_Spo        963  RGDEL---------------------------------ILSPN----------------------------  972
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Consensus aa:       pplp......ss@..............s..b.cs...................hpblbbb.pp.....
Consensus ss:       hhhhh    hhhhhhh            hh    h                 hhhhhhhhhhhhh


Conservation:
CWF4_Spo       674  A-------------------------------------------------------------------  674
SYF3_human     836  EHHPD-----------------------------------EDVDESES----------------------  848
CWF3_Spo       770  -----------AGFVLSKSNPQETSKITGEEN-------------------------------------  790
SYF1_human     784  ERDQPLRAQSKILFVRSDASREELAELAQQVNPEEIQLGEDEDEDEMDLEPNEVRLEQQSVPAAVFGSLK  853
NRDE2_Cele    1243  --------QEHSLFVTDEEYMKTQQEIGLQY------------SESGR--------------------- 1270
NRDE2_human   1149  LRVRLPL-EELELLLED----------------------------------------------------- 1164
NRL1_Spo            -------------------------------------------------------------------
Consensus aa:       ...................................................................
Consensus ss:


Conservation:
CWF4_Spo            --
SYF3_human          --
CWF3_Spo            --
SYF1_human     854  ED  855
NRDE2_Cele          --
NRDE2_human         --
NRL1_Spo            --
Consensus aa:       ..
Consensus ss:
```

Figure S2. NRDE-2 like proteins are structurally similar to Syf1 and Syf3 splicing factors. NRDE-2 proteins from *S. pombe* (Spo), *C. elegans* (Cele) and *H. sapiens* (human), and the Syf1 and Syf3 factors from human and S. pombe (Cwf3 and Cwf4), were aligned using PROMALS3D as described in Figure S1.

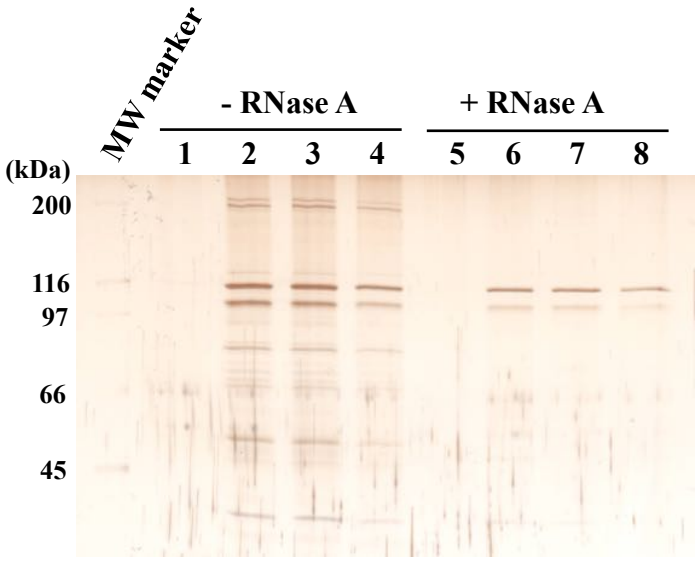

Figure S3. Silver staining of Nrl1 associated proteins. Protein complexes associated with Nrl1-TAP were isolated by tandem affinity purification. 10 µl of protein fraction eluates from purifications carried out without RNase A treatment (lane 1-4, - RNase A) or in presence of RNase A (lane 5-8, + RNase A) were separated by 8% SDS-PAGE and visualized by silver staining. Molecular weight markers (MW marker) are indicated on the left.

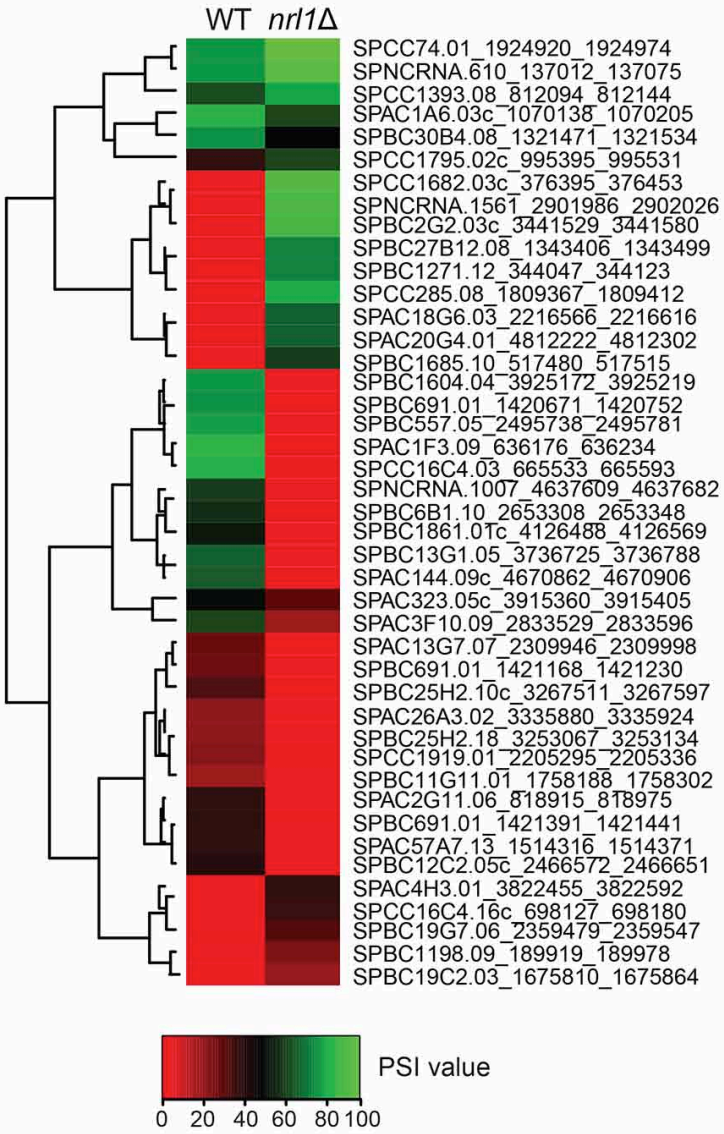

Figure S4. Nr1 strains display spicing defects. Heatmap of splicing changes by differences in the PSI values between WT and *nrl1Δ*. 43 genes displayed significant PSI differences between WT and *nrl1*∆. For the dendrogram shown, hierarchical clustering was performed using Euclidean distance. In each entry of the heatmap the gene and the genomic coordinates of the respective intron are depicted (see Table S2 for details). The heatmap was built using the gplots R package (heatmap.2 function).

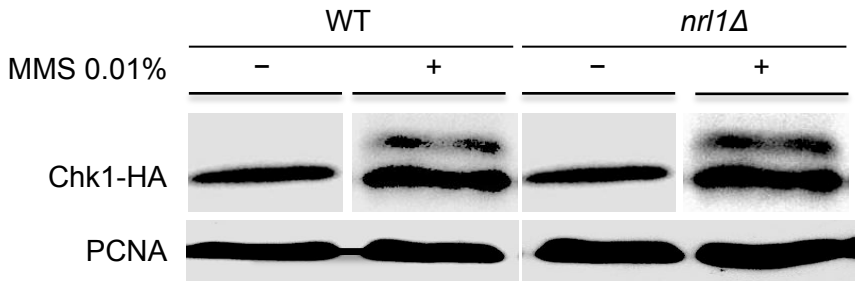

Figure S5. *nrl1*Δ cells maintain a functional DNA damage checkpoint. WT and *nrl1*Δ strains harboring a Chk1 protein tagged with Hemagglutinin (HA) at OD=0.3 were grown for 4 hours in YE6S in presence (+) or absence (-) of MMS (0.01%). Levels of phosphorylated Chk1 protein were analyzed by western blot using the mouse monoclonal anti-HA antibody 12CA5 (Abcam). Activation of Chk1 results in a slower migrating species because of phosphorylation of S345 by Rad3.

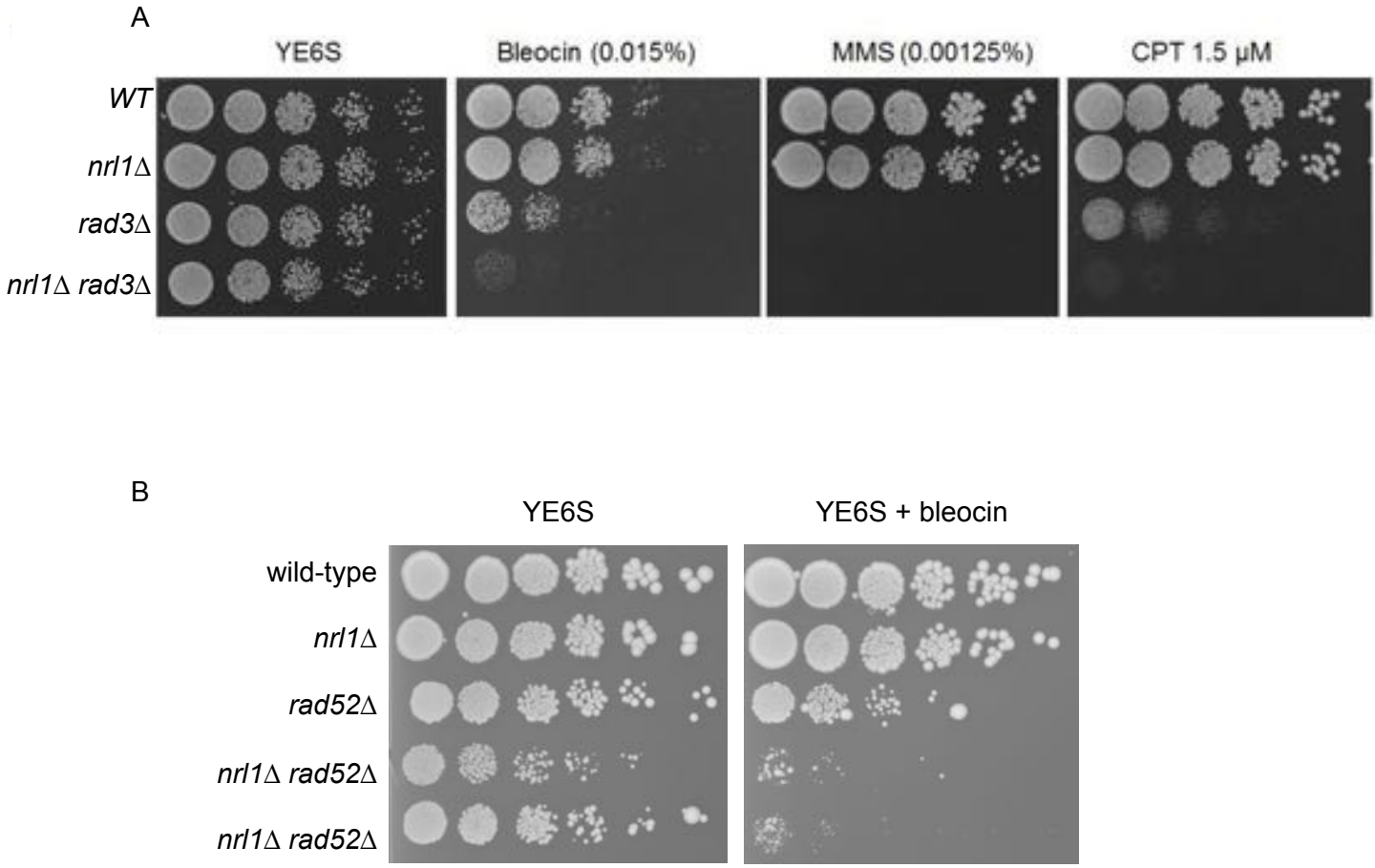

Figure S6. *nrl1Δ rad3Δ* and *nrl1Δ rad52Δ* strains are hypersensitive to low levels of genotoxic agents. (A) Fivefold serial dilutions of WT (TH2094) and *nrl1Δ* (TH8103), *rad3Δ* (TH585) and *nrl1Δ rad3Δ* (TH8127) on YE6S, methyl methanesulfonate (MMS), bleomycin (Bleo) and camptothecin (CPT) at indicated concentrations. (B) Fivefold serial dilutions of WT (TH2093) and *nrl1Δ* (TH8340), *rad52Δ* (TH2129) and *nrl1Δ rad52Δ* (TH8698-9) on YE6S, and YE6S with 0.2µg/ml bleocin.

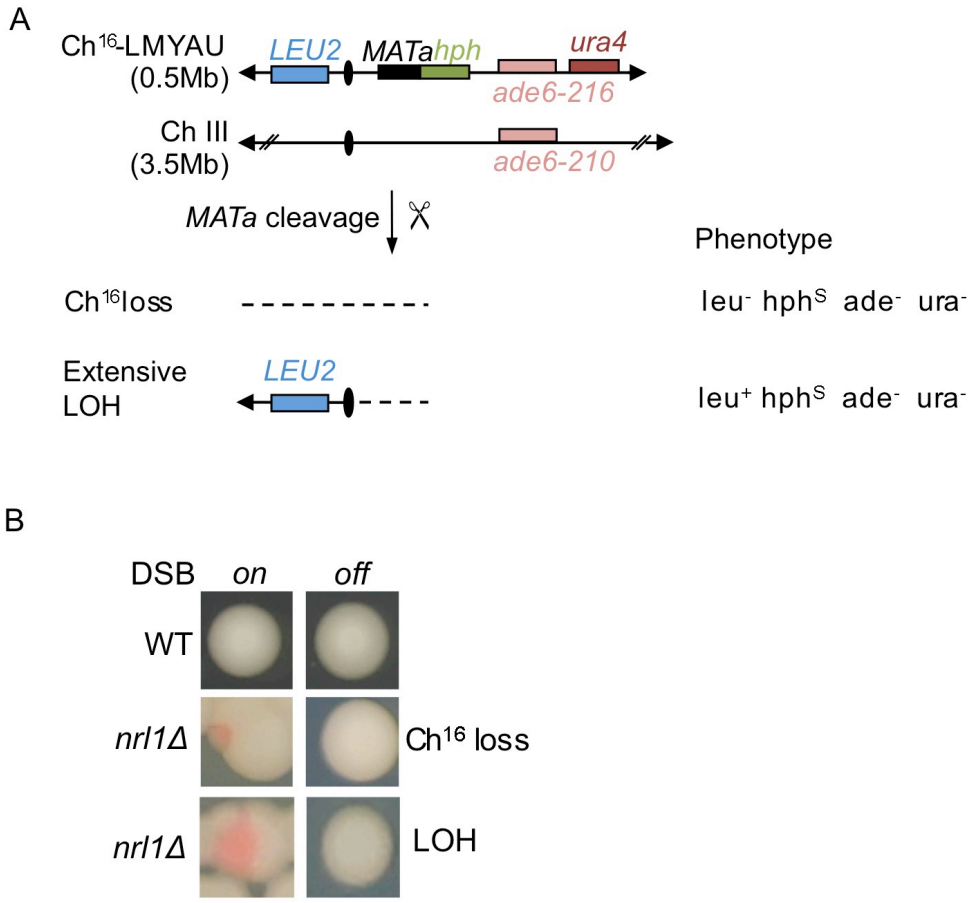

Figure S7 Loss of Nrl1 results in break-induced chromosome loss and chromosomal rearrangements. (A) Schematic of the minichromosome Ch^{16}-LMYAU and outcomes leading to increased break-induced colony sectoring. Derepression of HO endonuclease (by removal of thiamine) generates a DSB at the *MAT*a target site (indicated by scissors). (B) Colony sectoring DSB assay of *nrl1Δ* (TH8103). Sectoring resulting from break-induced Ch^{16} loss (indicated) was detected on EMM plus leucine, uracil and low adenine (5 mg/L) while sectoring resulting from break-induced LOH (indicated) was detected on EMM plus uracil and low adenine (5 mg/L) in the presence (DSB off) or absence (DSB on) of thiamine.

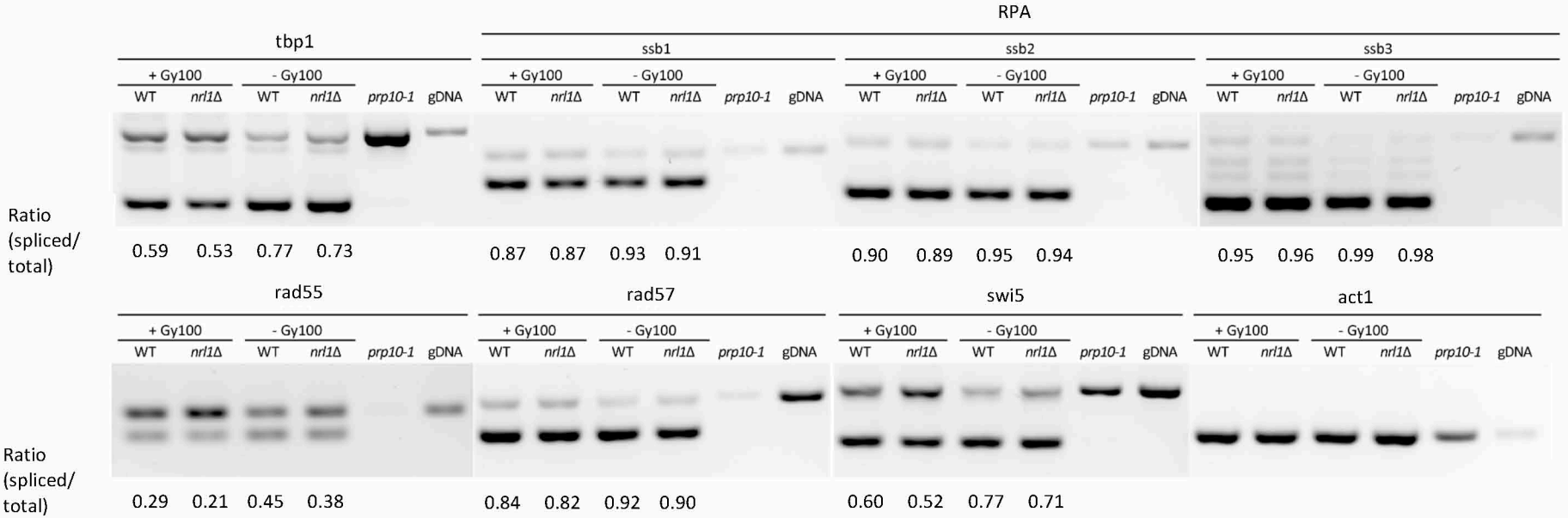

Figure S8. *nrl1+* deletion does not affect the splicing of HR factors shown acting upstream of Rad51. Total RNA was isolated from non-irradiated (-Gy100) and irradiated (+Gy100) wildtype (WT) and nrl1Δ cells, treated with DNase I (Roche), and reverse transcribed (OmniScript, Qiagen). PCR was carried out with primers designed to span at least one exon. As controls, total RNA from a splicing factor mutant (prp10-1) and genomic DNA were used. Ratios between the spliced and total (spliced+unspliced) products are presented. Band intensities were measured using ImageJ, (http://imagej.nih.gov/ij/). ssb1-3 indicate the three subunits of RPA; tbp1 (TATA-binding-protein 1) : non-HR control; act1: loading control.

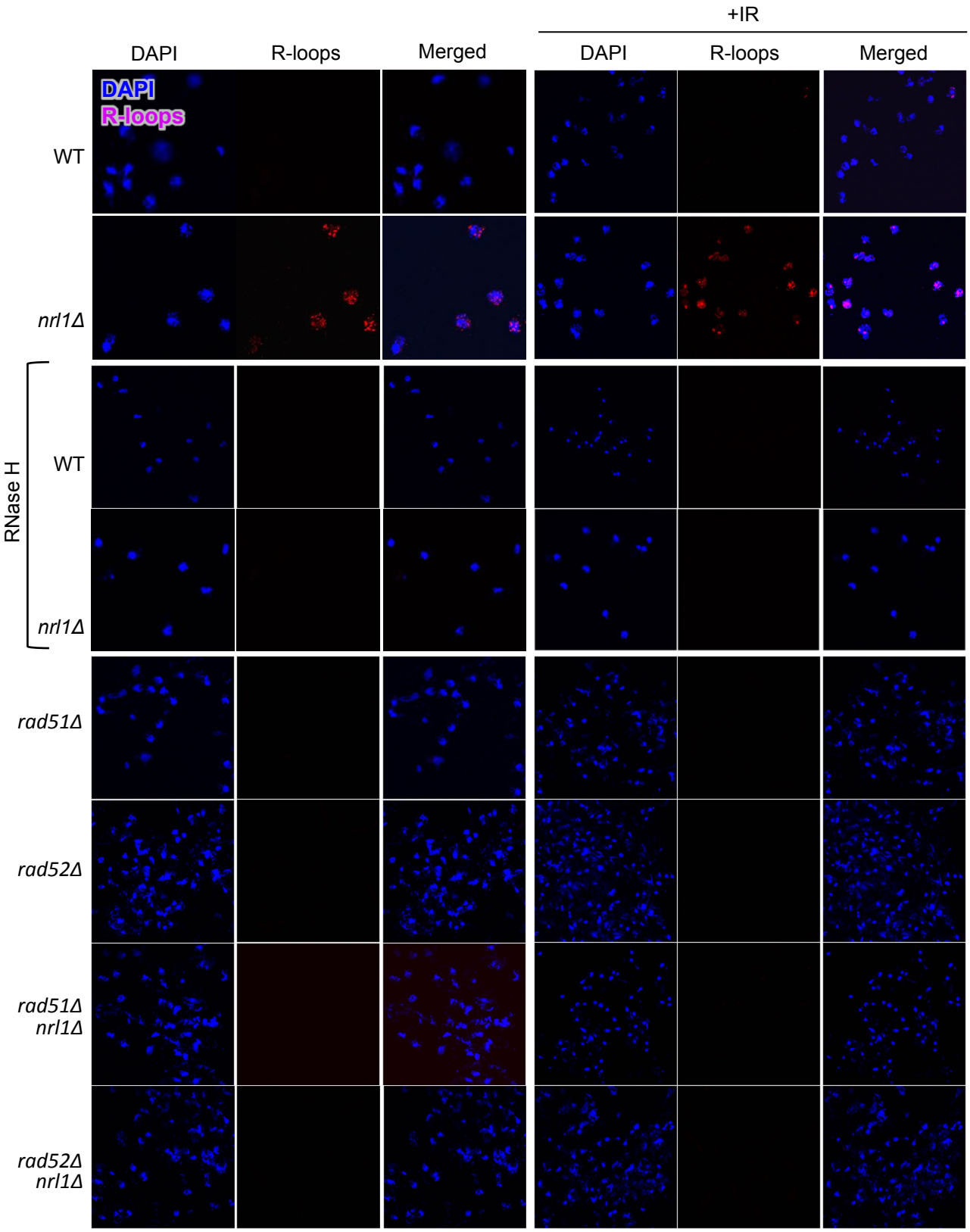

Figure S9. R-loop accumulation in WT, *nrl1Δ, rad51Δ, rad52Δ, rad51Δ nrl1Δ* and *rad52Δ nrl1Δ* prior and upon IR exposure. Chromosome spreads were immunostained using the S6.9 antibody as previously described [52]. As negative control, the spreads were pre-treated with RNase H (+ RNAse H) before immunostaining. +IR: The cells were exposed to 100 Gy of IR before immunostaining.

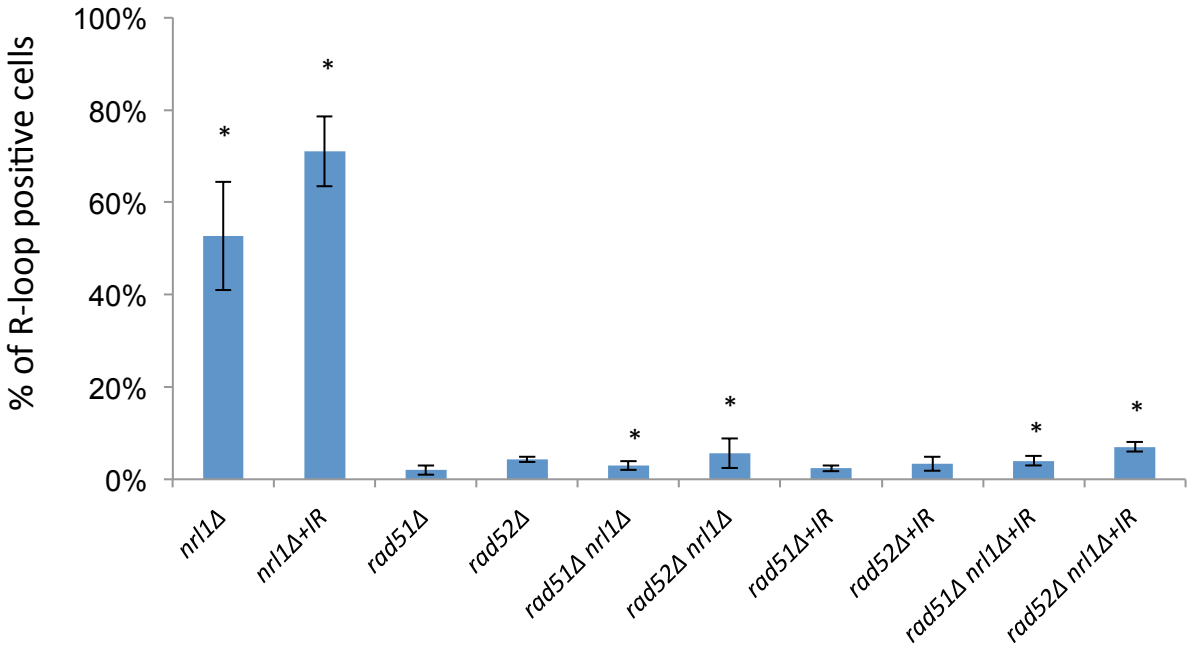

Figure S10. Quantification of R-loop positive nuclei in WT, *nrl1Δ, rad51Δ, rad52Δ, rad51Δ nrl1Δ and rad52Δ nrl1Δ* strains before and after IR exposure. +IR: The cells were exposed to 100 Gy of IR and incubated for 4 hrs before immunostaining. Mean and standard deviation were scored from triplicate experiments, n > 200. The asterisks (*) indicate significant differences compared with WT as determined by paired T-test ($p \leq 0.05$). +IR: The cells were exposed to 100 Gy of IR before immunostaining.

Supplemental methods and detailed protocols

Protein purification

Cells expressing Nrl1-TAP were grown in 16 L of complete yeast extract medium (YE+5S) to mid-log phase (OD ~0.8). 8 L of culture was concentrated to 300 mL and exposed to 100 Gy of gamma irradiation (+IR, 3.3 Gy/min) in a 500 ml conical, for a total of 30 min. After irradiation cells were resuspended in 8 L of YE+5S medium and recovered for 30 min at 30°C. The same procedure was applied for control cells (-IR), except irradiation step. Following, cells were harvested by centrifugation and Nrl1-TAP associated proteins were isolated. Briefly, yeast cell powder was made using SPEX SamplePrep 6870 Freezer/Mill. Proteins were extracted using IPP150 buffer (1 g of powder per 3 mL of IPP150 buffer) (IPP150, 50 mM Tris pH 8.0, 150 mM NaCl, 10% glycerol, 0.1% NP-40, supplemented with complete protease and phosphatase inhibitors and 1 mM PMSF). 500 µL of IgG beads was incubated with protein extracts for 2 h at 4°C. Beads were washed with 20 volumes of IPP150 buffer, with 5 volumes of TEV cleavage buffer A (TCBA, 10 mM Tris pH 8.0, 150 mM NaCl, 10% glycerol, 0.1% NP-40, 0.5 mM EDTA), resuspended in 2 mL of TCBA supplemented with RNase A (5 µg/mL) and incubated for 30 min at 16°C. Following RNase A treatment, the samples were supplemented with DTT (1 mM) and the AcTEV™ protease (400 units) was added. The TEV cleavage step was performed for 2 h at 16°C. The eluate was supplemented with 6 µl of 1 M CaCl2, mixed with 6 ml of Calmodulin binding buffer 1 (CBB1, 10 mM Tris pH 8.0, 150 mM NaCl, 10% glycerol, 0.1% NP-40, 1 mM imidazole, 1 mM Mg-acetate, 2 mM CaCl2, 10 mM β-mercaptoethanol), 200 µL of calmodulin beads and incubated for 2 h at 4°C. The beads were washed with 10 volumes of CBB1 and 5 volumes of Calmodulin binding buffer 2 (CBB2, 10 mM Tris pH 8.0, 150 mM NaCl, 1 mM Mg-Acetate, 2 mM CaCl2, 1 mM β-mercaptoethanol). The proteins were step-eluted using 200 µL of elution buffer (10 mM Tris pH 8.0, 150 mM NaCl, 1 mM Mg-acetate, 2 mM EGTA, 1 mM β-mercaptoethanol). Eluted proteins were separated on SDS-PAGE and visualized by silver staining. Eluates from peak fractions were combined and submitted for LC-MS/MS analysis.

Enzymatic digest, LC-MS/MS analysis and data analysis

The pH of the eluted protein sample was adjusted to 8.5, disulfide bonds were reduced with DTT and subsequently alkylated with iodoacetamide . Proteins were digested with trypsin (recombinant, proteomics grade, Roche; 1:25 of the estimated

amount of protein) at 37°C overnight and stopped by addition of trifluoroacetic acid (TFA) to approx. pH 2. Digests were separated on an UltiMate 3000 RSLCnano LC system (Dionex, Thermo Fisher Scientific). Peptides were loaded onto a trapping column (PepMap C18, 5µm particle size, 300 µm i.d. x 5mm, Dionex, Thermo Fisher Scientific) equilibrated with 0.1% TFA and separated on an analytical column Acclaim PepMap RSLC C18, 50 cm × 75 µm × 2 µm, 100 Å, Dionex, Thermo Fisher Scientific) applying a 60 minutes linear gradient from 1.6% up to 30% acetonitrile (ACN). The HPLC (nano RSLC from Dionex, Thermo Fisher Scientific) was directly coupled to a QExactive mass spectrometer (Thermo Fisher Scientific) via a nanoelectrospray ionization source (Proxeon, Thermo Fisher Scientific). The electrospray voltage was set to 1900 V. The QExactive mass spectrometer was operated in the data-dependent mode: 1 full scan (m/z: 350-1650, resolution 70000) with lock mass enabled was followed by maximal 12 MS/MS scans. The lock mass was set at the signal of polydimethylcyclosiloxane at m/z 445.120025. The 12 most intense ions were fragmented by higher energy collisional dissociation (HCD) with normalized collision energy of 30. Fragment spectra were acquired with a resolution of 17500. The ion target value for full MS was set to 1,000,000, for MS/MS to 100,000. Fragmented ions were excluded from further selection for 30 s. Raw data were searched with MaxQuant 1.5 against the S. pombe DB (5144 entries, 2013-05-09, http://www.pombase.org/) including the contaminant collection with the following parameters: trypsin was selected as protease allowing two missed cleavages, carbamidomethylcysteine was set as static modification, oxidation of methionine, phosphorylation of serine, threonine and tyrosine as well as protein N-terminal acetylation as variable modifications. Precursor tolerance was set to 4.5 ppm and 20 ppm MS/MS for the fragment tolerance. Results were filtered at the peptide and at the protein level to 1% FDR. Relative quantification was based on the total peak intensities for each protein calculated with MaxQuant. For comparison protein intensities were normalized to the intensity of Nrl1 in proliferating cells.