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Supplementary information

Structural basis of catalytic activation in human splicing

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SI Guide

Structural basis of catalytic activation in human splicing

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SI Figure 4 | Sequence alignment of PRP2, SKIP, and PPIL4 orthologs. The sequences of $PRP2^{NTD}$, the molecular brake's SKIP region, and the full-length PPIL4 are shown. *Hs – Homo sapiens, Ce – Caenorhabditis elegans, At – Arabidopsis thaliana, Sp – Schizosaccharomyces pombe, Sc – Saccharomyces cerevisiae.*

SI Video 1. Overview of the cryo-EM density of the B^{AQR} spliceosome. The overall density is shown in grey and the densities of the core and peripheric modules are colored.

SI Video 2. Translocation of PRP2 along the intron and SF3B1's opening on the branch duplex, at the transition from B^{act} to B^{AQR} complexes.

SI Video 3. Remodeling of the spliceosome by PRP2's translocation, at the B^{act} to B^{AQR} transition. The movie depicts the stepwise destabilization of subunits followed by the assembly of the molecular brake that terminates PRP2's translocation.

SI Video 4. Remodeling of B^{AQR} at the transition to B^{*}/C complexes. The movie depicts the destabilization of various subunits present in B^{AQR} and the relocation of the branch duplex to the active center of the spliceosome for the branching reaction.

SI Video 5. Termination of PRP2's translocation by the construction of a molecular brake. PPIL4, SKIP, and $PRP2^{NTD}$ bind the exiting intron-like a shoe brake, while SKIP's wedge element intercalates between the RecA-like and CTD domains of PRP2, immobilizing the helicase in the open conformation. PRP2 in the open conformation (after ATP hydrolysis and translocation) corresponds to the B^{AQR} structure. The closed conformation is modeled based on the crystal structure of Prp2 from *Chaetomium thermophillum*, in complex with ADP-$BeF3^{-}$ and a poly-U_{12} RNA (PDB 6zm2).

Caption for Data S1. Protein composition of purified human B^{AQR} spliceosomes as revealed by mass spectrometry analyses.

Caption for Data S2. Model building details for the B^{AQR} complex. The B^{AQR} subunits/regions modeled only in the overall maps M5 and M4 (corresponding to states A and B) are colored in purple.

Figure 1

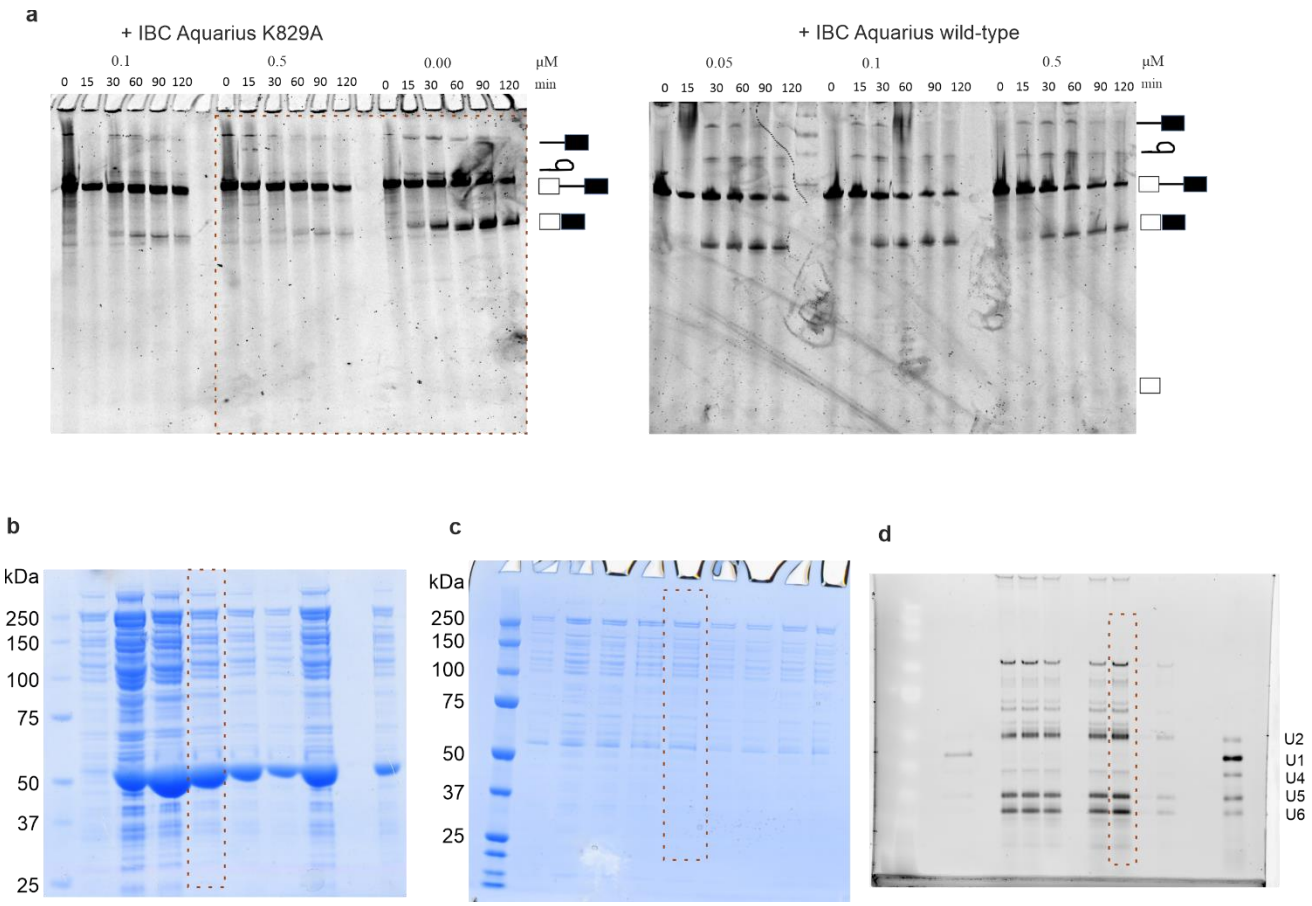

a
+ IBC Aquarius K829A
0.1
0.5
0.00
µM
0 15 30 60 90 120 0 15 30 60 90 120 0 15 30 60 90 120
min
b
+ IBC Aquarius wild-type
0.05
0.1
0.5
µM
0 15 30 60 90 120 0 15 30 60 90 120 0 15 30 60 90 120
min
b
b
kDa
250
150
100
75
50
37
25
c
kDa
250
150
100
75
50
37
25
d
U2
U1
U4
U5
U6

Figure 2

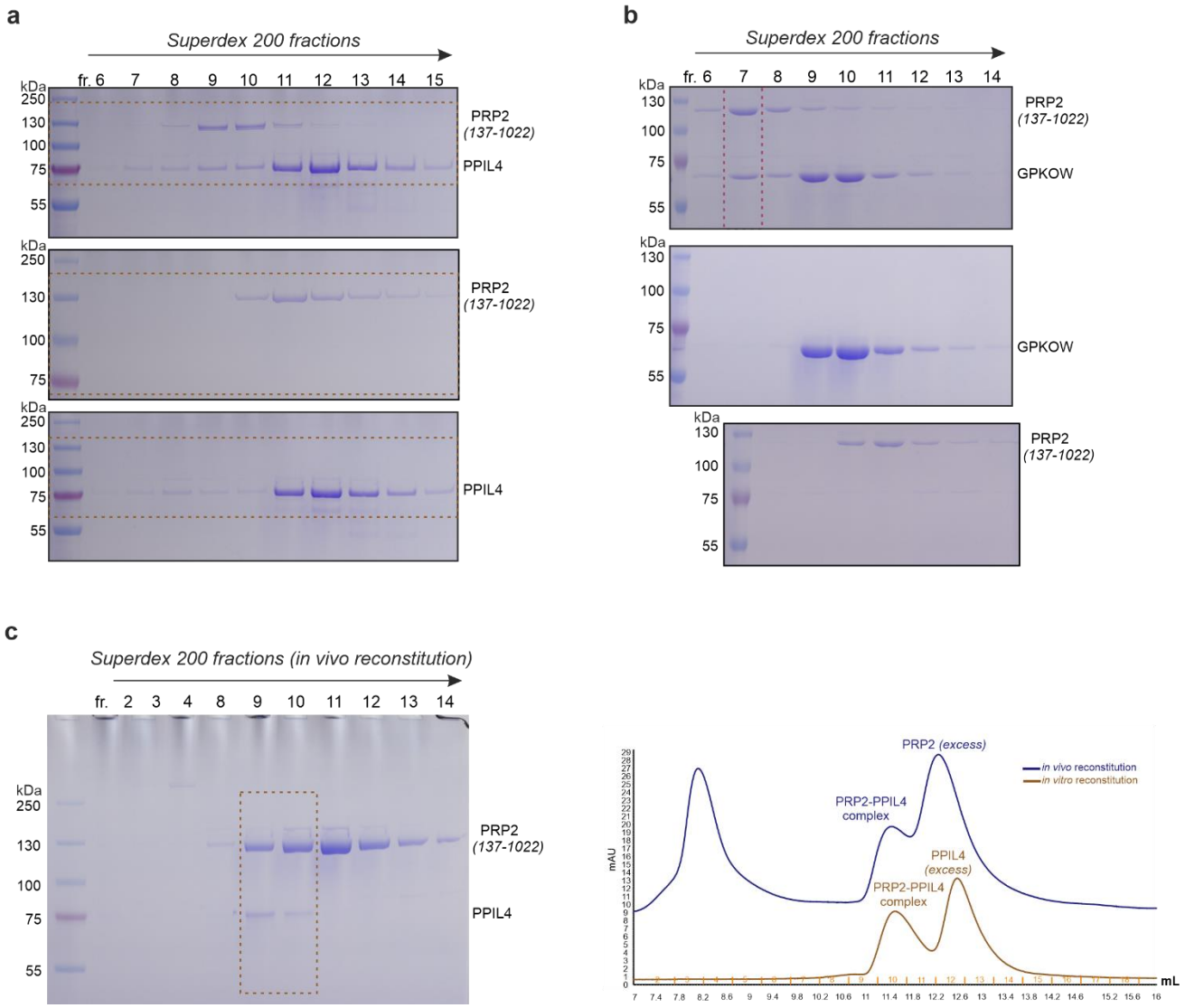

a
Superdex 200 fractions
fr. 6 7 8 9 10 11 12 13 14 15
kDa
250
130
100
75
55
PRP2
(137-1022)
PPIL4
b
Superdex 200 fractions
fr. 6 7 8 9 10 11 12 13 14
GPKOW
c
Superdex 200 fractions (in vivo reconstitution)
fr. 2 3 4 8 9 10 11 12 13 14
PRP2 (excess)
in vivo reconstitution
in vitro reconstitution
PRP2-PPIL4
complex
PPIL4
(excess)
mAU
mL

Figure 3

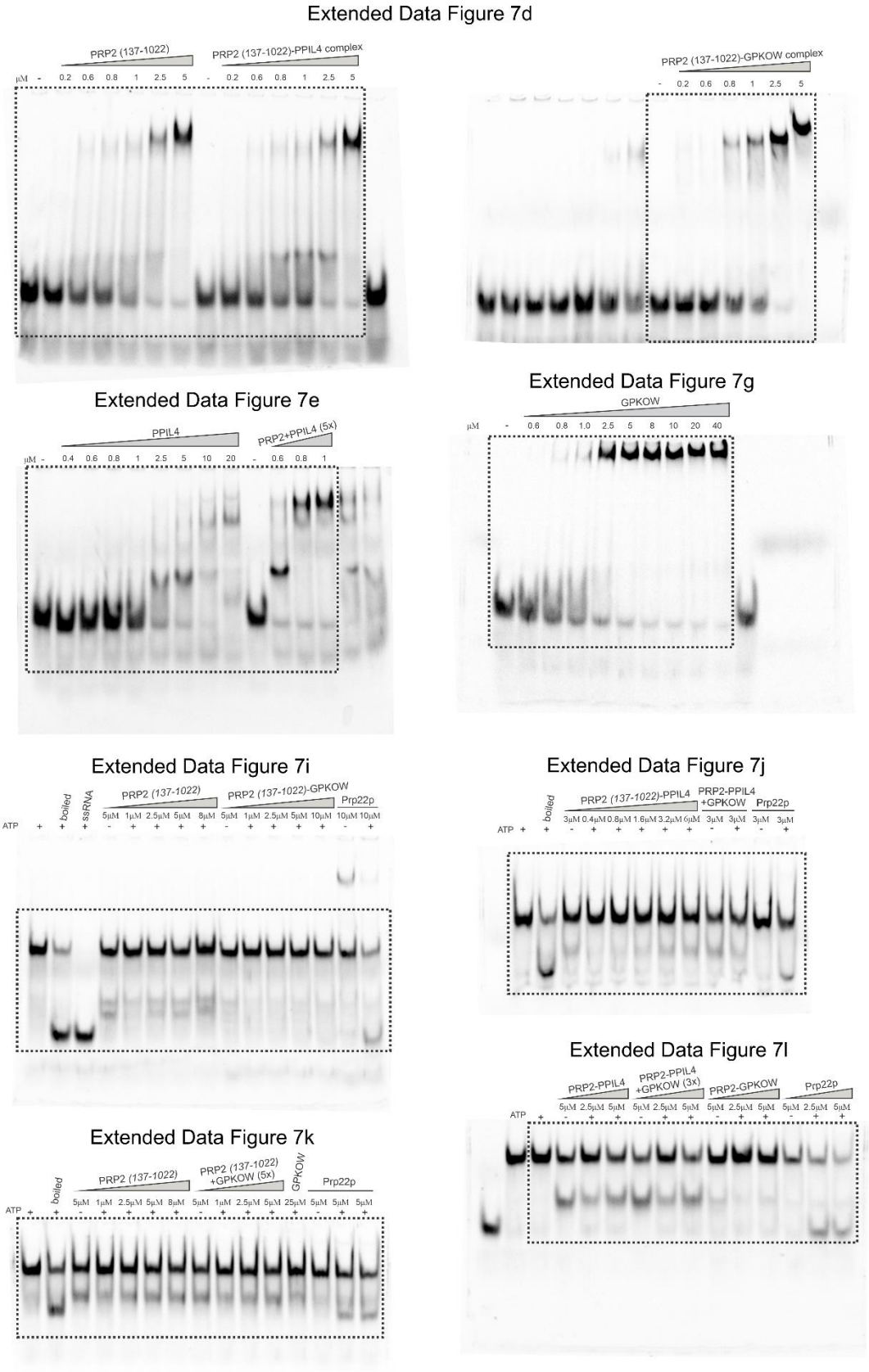
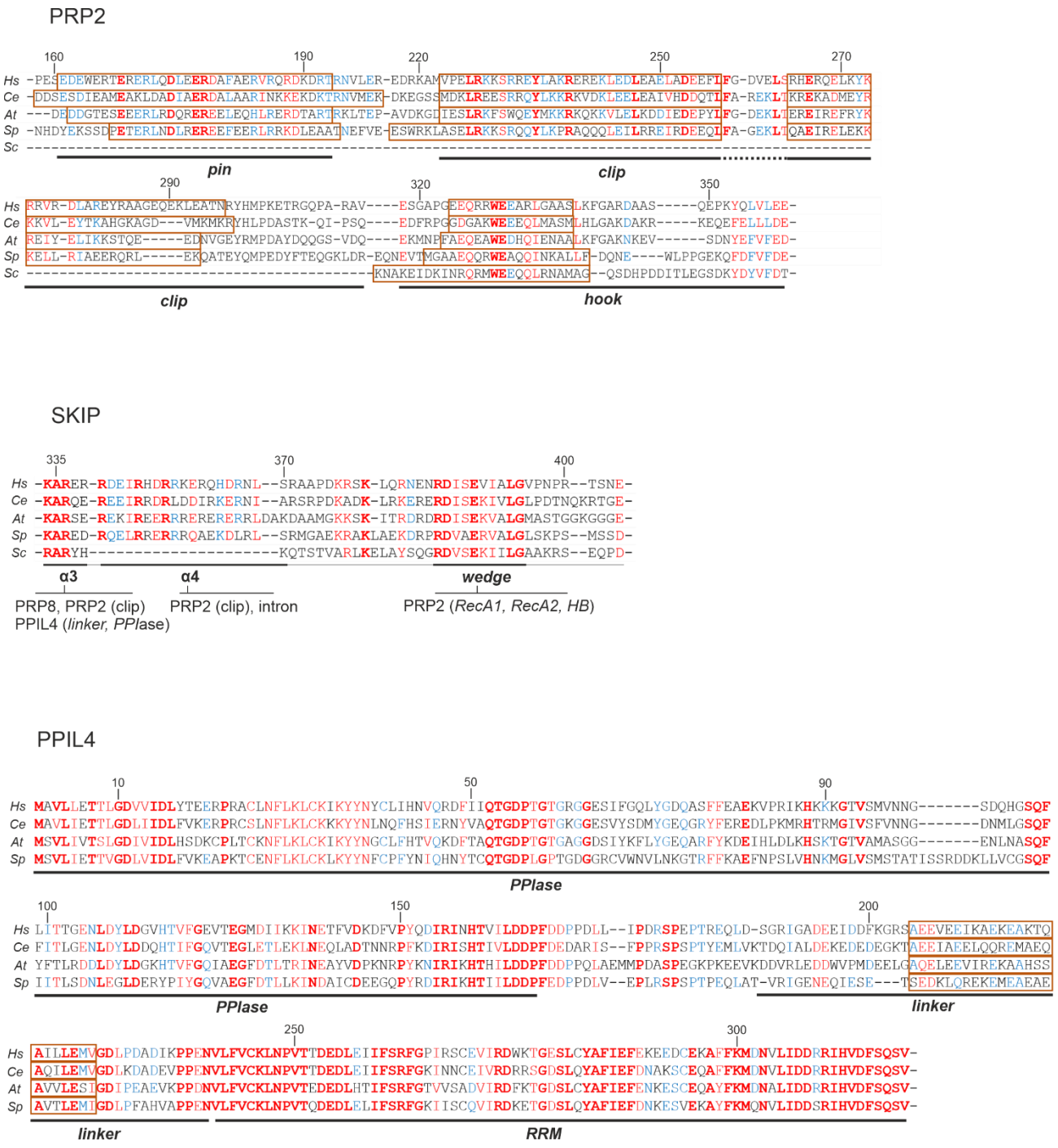

Extended Data Figure 7d
PRP2 (137-1022)
PRP2 (137-1022)-PPIL4 complex
PRP2 (137-1022)-GPKOW complex
Extended Data Figure 7e
PPIL4
PRP2+PPIL4 (5x)
Extended Data Figure 7g
GPKOW
Extended Data Figure 7i
boiled
ssRNA
PRP2 (137-1022)
PRP2 (137-1022)-GPKOW
Prp22p
ATP
Extended Data Figure 7j
PRP2 (137-1022)-PPIL4
PRP2-PPIL4 +GPKOW
Prp22p
Extended Data Figure 7k
PRP2 (137-1022)
PRP2 (137-1022) +GPKOW (5x)
GPKOW
Prp22p
Extended Data Figure 7l
PRP2-PPIL4
PRP2-PPIL4 +GPKOW (3x)
PRP2-GPKOW
Prp22p

Figure 4


PRP2
160
190
220
250
270
Hs -PESEDEWERTERERLQDLEERDAFAERVRQRDKDRTRNVLER-EDRKAMVPELRKKSRREYLAKREREKLEDLEAELADEEFIFG-DVELSRHERQELKYK
Ce -DDSESDIEAMEAKLDADIAERDALAARINKKEKDKTRNVMEK-DKEGSSMDKLREESRRQYLKKRKVDKLEELEAIVHDDQTIFA-REKLTKREKADMEYR
At ---DEDDGTESEEERLRDQRERERELEQHLRERDTARTRKLTEP-AVDKGDIESLRKFSWQEYMKKRKQKKVLELKDDIEDEPYIFG-DEKLTERERIREFRYK
Sp -NHDYEKSSDPETERLNDLRERERFEERLRRKDLEAATNEFVE-ESWRKLASELRKKSRQQYLKPRAQQQLEILRREIRDEEQIFA-GEKLTQAEIRELEKK
Sc ----------
pin
clip
290
320
350
Hs RRVR-DLAREYRAAGEQEKLEATNRYHMPKETRGQPA-RAV----ESGAPGEEQRRWEEARLGAASLKFGARDAAS-----QEPKYQLVLEE-
Ce KKVL-EYTKAHGKAGD---VMKMKRYHLPDASTK-QI-PSQ----EDFRPGGDGAKWEEEQLMASMLHLGAKDAKR-----KEQEFELLLDE-
At REIY-ELIKKSTQE-----EDNVGEYRMPDAYDQQGS-VDQ----EKMNPFAEQEAWEDHQIENAALKFGAKNKEV------SDNYEFVFED-
Sp KELL-RIAEERQRL-----EKQATEYQMPEDYFTEQGKLDR-EQNEVTMGAAEQQRWEAQQINKALLF-DQNE----WLPPGEKQFDFVFDE-
Sc ----------KNAKEIDKINRQRMWEEQQLRNAMAG--QSDHPDDITLEGSDKYDYVFDT-
clip
hook
SKIP
335
370
400
Hs -KARER-RDEIRHDRRKERQHDRNL--SRAAPDKRSK-LQRNENRDISEVIALGVPNPR--TSNE-
Ce -KARQE-REEIRRDRLDDIRKERNI--ARSRPDKADK-LRKERERDISEKIVLGLPDTNQKRTGE-
At -KARSE-REKIREERRRERERERRLDAKDAAMGKKSK-ITRDRDRDISEKVALGMASTGGKGGGE-
Sp -KARED-RQELRRERRRQAEKDLRL--SRMGAEKRAKLAEKDRPRDVAERVALGLSKPS--MSSD-
Sc -RARYH----------KQTSTVARLKELAYSQGRDVSEKIILGAAKRS--EQPD-
α3
α4
wedge
PRP8, PRP2 (clip)
PPIL4 (linker, PPIase)
PRP2 (clip), intron
PRP2 (RecA1, RecA2, HB)
PPIL4
10
50
90
Hs MAVLLETTLGDVVIDLYTEERPRACLNFLKLCKIKYYNYCLIHNVQRDFIIQTGDPTGTGRGGESIFGQLYGDQASFFEAEKVPRIKHKKKGTVSMVNNG-------SDQHGSQF
Ce MAVLIETTLGDLIIDLFVKERPRCSLNFLKLCKKKYYNLNQFHSIERNYVAQTGDPTGTGKGGESVYSDMYGEQGRYFERELDLPKMRHTRMGIVSFVNNG-------DNMLGSQF
At MSVLIVTSLGDIVIDLHSDKCPLTCKNFLKLCKIKYYNGCLFHTVQKDFTAQTGDPTGTGAGGDSIYKFLYGEQARFYKDEIHLDLKHSKTGTVAMASGG-------ENLNASQF
Sp MSVLIETTVGDLVIDLFVKEAPKTCENFLKLCKLKYYNFCPFYNIQHNYTCQTGDPLGPTGDGGRCVWNVLNKGTRFFKAEFNPSLVHNKMGLVSMSTATISSRDDKLLVCGSQF
PPIase
100
150
200
Hs LITTGENLDYLDGVHTVFGEVTEGMDIIKKINETFVDKDFVPYQDIRINHTVILDDPFDDPPDLL--IPDRSPEPTREQLD-SGRIGADEEIDDFKGRSAEEVEEIKAEKEAKTQ
Ce FITLGENLDYLDDQHTIFGQVTEGLETLEKLNEQLADTNNRPFKDIRISHTIVLDDPFDEDARIS--FPPRSPSPTYEMLVKTDQIALDEKEDEDEGKTAEEIAEELQQREMAEQ
At YFTLRDDLDYLDGKHTVFGQIAEGFDTLTRINEAYVDPKNRPYKNIRIKHTHILDDPFDDPPQLAEMMPDASPEGKPKEEVKDDVRLEDDWVPMDEELGAQELEEVIREKAAHSS
Sp IITLSDNLEGLDERYPIYGQVAEGFDTLLKINDAICDEEGQPYRDIRIKHTIILDDPFEDPPDLV--EPLRSPSPTPEQLAT-VRIGENEQIESE---TSEDKLQREKEMEAEAE
PPIase
linker
250
300
Hs AILLEMVGDLPDADIKPPENVLFVCKLNPVTTDEDLEIIFSRFGPIRSCEVIRDWKTGESLCYAFIEFEKEEDCEKAFKMDNVLIDDRRIHVDFSQSV-
Ce AQILEMVGDLKDADEVPPENVLFVCKLNPVTTDEDLEIIFSRFGKINNCEIVRDRRSGDSLQYAFIEFDNAKSCEQAFFKMDNVLIDDRRIHVDFSQSV-
At AVVLESIGDIPEAEVKPPDNVLFVCKLNPVTEDEDLHTIFSRFGTVVSADVIRDFKTGDSLCYAFIEFENKESCEQAYFKMDNALIDDRRIHVDFSQSV-
Sp AVTLEMIGDLPFAHVAPPENVLFVCKLNPVTQDEDLELIFSRFGKIISCQVIRDKETGDSLQYAFIEFDNKESVEKAYFKMQNVLIDDSRIHVDFSQSV-
linker
RRM