

A

*hsSpt6* MSDFVSEAEES...EEEYNDEGEVVPRTKKF.VEEEDDDEEEEEEENLDDQDEOQNL 54  
*scSpt6* MEETGDSKLVPRDEEEIIVNDNDETAKPSEEEEGEDVFD...SEEDDIDEDDEDEARKV 56  
*ecSpt6*  
*paTex*

*hsSpt6* .KGFINDDDDEDEGEDEEGSDSGDSEDDVGHKKRKRRTSFDDRLEDDDFDLIEENLG...V 110  
*scSpt6* QEGFIVNDDDENEDPG...TSIS..KKRRKHKRREREE.DDRLEDDDLMLMENAGVERT 110  
*ecSpt6*  
*paTex*

*hsSpt6* KVKRQOK.YRRVKKMSDDEDDDEEEYGKEEHEKEAIA...EEIFQDGESEE...GQ 159  
*scSpt6* KASSSSGKFKRLLKRVGDEGNAAESESNDVAASRQDSTSKLEDFPSEDEEEESGLRNGRN 170  
*ecSpt6*  
*paTex*

*hsSpt6* EAMEAPMAPPEEEEE...DDEESDIDDDFIIVDD...DGQPLKKPKW 198  
*scSpt6* NEYGRDEEDHENRRRTADKGGILDELDDDFIEDDEFSDDEDETRQRRIQEKKLLREQSIKQ 230  
*ecSpt6* MGDSSSEEVINISRREHQVARRRVLEDLFEEDEREISE 38  
*paTex*

*hsSpt6* RKKLPGYTDAAALQEAQEIFGVDVDFDYDEFEKYNEYDELEEEYEDDEAEGEIRVRPKKT 258  
*scSpt6* PTQITGLSSDKIDEMYDIFGDDGHDYDWALEIENEDEIENGNDNNEAE...IDEETGA 286  
*ecSpt6* ESISSIDYGD RDSIFFEIFGTGEERYVLESDFHEVAE... 76  
*paTex*

**Spt6N**

*hsSpt6* TKKRVSRRSIFEMYEPSELESSHLTDQDNEIRATDLPERFQLRSIPV...KGAED..DEL 313  
*scSpt6* IKSTKKKISLQDIYDLEDDLKKNLMTTEGDMKIRKTDIPERYQELRAGITDYGNMSSSEDQEL 346  
*ecSpt6* .. 76  
*paTex*

*hsSpt6* EEEADWIYRNAFATPTISLQESCDYLDRGQPASSFSRKGPSSTIQKIKKEALGFMR..NQHF 371  
*scSpt6* ERN..WIAEKISVDKNFDANYDLTE...FKEATIGNAIKFIIT..KENL 386  
*ecSpt6* ..EAGRCDEPTPEKTVDL...DKVYEAANNMRTRTESLKKKIELV 118  
*paTex* MDSINTRIAEELSALPSSGRVQPQQVAAAVALL...DEGSTVFP 40

**Hth**

*hsSpt6* EVPFIATFYRKEY...VEPELHINDLWRVWQWDEKWTQLRIRKENLTRLFEKMQAYQY 425  
*scSpt6* EVPFIAYYRRNYISSREKDGFLLEDLWVVSVDIEFHSLVNKKDYVQRFFYAE... 441  
*ecSpt6* GRGYTIHFALLHSG...EDVGIEE.GYEVVDIFDEYREFVDLKEKMRRRYGNDK... 169  
*paTex* IARYRKEV...TG.SLDDTQLRMLLEERLRYLRELEERRGAILASTIEQ...KLT 88

*hsSpt6* EQISADPDK.PLADGIRALDITDMERLKDVOQSMDELKDVYNHFLLYYGRDIPKMQNAAKA 484  
*scSpt6* HEDDPVIT.EYFKN...QNTASIAELNSLQDIYDYLEFKYANEINE.MFINHT 489  
*ecSpt6* LLLQDIEGIQ...GLKWYSAYKKHL... 190  
*paTex* PELAR.DIKLA...DTKTRLEDLILPY... 111

*hsSpt6* SRKKLKRVRREEGDEEGEGDEAEDEEQRGPELQASRRDMYTTICQSAGLDGLAKKFGLTPE 544  
*scSpt6* GKTG...KHLKNSSYEKFKASPLYQAVSDIGISAE 522  
*ecSpt6* ...DKDLSLPEDELL... 201  
*paTex* ...KQRRTKQIALAEAGLGALADAL... 134

*hsSpt6* QFGENLRDSYQRHE.TEQFPAEPELELAKDYVCS...QFPTPEAVLEGARYMVALO 596  
*scSpt6* DVGENISSQHQIHPPVDHPSSKPVVEVIESILNANSGLDQVFTSNTKLAIDTVQKYYSLE 582  
*ecSpt6* ...SAEDFCENIKAKKKIHEP...RNSFLSDFANEDKLIQR 238  
*paTex* ...FDDPTLVPESEAAARFVDAEK...GFADV KAVLEGAKYILMERFAEDA 178

*hsSpt6* AREPLVRQVLRQTFQERAKLNTTPTKKGGRKDVDEAHY..AYSEKYALKNKPVKELRDDQ. 652  
*scSpt6* SKNTKIREKVRSDFFSKYLYLADVVLTAAGKKEFOKGS..YEDIKYALNRTPM.HFRDPPD 639  
*ecSpt6* SSLHPVRSALYTVFRNHG...IRSEKNGLDVRRDDVMK.RDLVDFVLSDISS... 285  
*paTex* TLLDCLKRVFMKNEATLTARVVP.G.KELEGARFSDYFEHDEPLKSGAPSHRA...LA 229

*hsSpt6* .FLKICLAEDGLLTTDISIDLKG...VEGYNDQTYFEEIKQFYR.DEFSHQVQEWNR 707  
*scSpt6* VFLKMEVAESLNLLSVKLHMSSQA...QYIEHLFQIALETTNTSDIAIEWNR 688  
*ecSpt6* ...ED...PWN 291  
*paTex* IFRGRNEGVLASLKVGG...EEAPGTLHPSEVMIAER..FGLS...NQGRAADKWLAE 279

*hsSpt6* QRTMAIERALQQFLYVOMAKELKNKLLAEAEKEYVIKACSRKLYNWRVAPYRPDQQVEED 767  
*scSpt6* FRKLAFNQAMDKIF.QDTSQEVKDNLTKNCKQLVAKTVRHKFMITKLDQAPPI... 740  
*ecSpt6* YRERIIRSSAEKAAKEAVPLVVKETDLREMK...RRVFVEAVDRIVNGSIGIK... 342  
*paTex* VVRWTKVKKLYTHLETDLFGELELDGAEDEAISVFARNLHDLLELAAPAGPR... 329

hsSpt6 22DFMDENOGKGIKIVLGIATFSSAR.DH.PVFCALVNGEGETVDFLRLPHFTRKRRTAWREEE 825  
scSpt6 ...NVRDPKPKIKLSLTTCGQGRFAGADAFVYVNRKGDFFIRDYKI...VDNPPDKNTN 791  
ecSpt6 ...GDGTGCVGVTREKKY...LKAFTVNFEGDLDVYITV...KEEE 379  
paTex ...ATLGLDFGLRT...GKVKVAVDATTGRLDATTV...YFHAPKKNQWDC 370

hsSpt6 REKKAQDIETLKKEFLNKKPHVTVAG...ENRDAQMLIEDVKRIVHELD.QGQQLSSIGV 882  
scSpt6 PEKF...EDTLDNIQSCOPNAVING...PNPKTKFYKRLQEVLHKKQIVDSRGTIPI 846  
ecSpt6 K...DLRLRFTEKFDPCVAVSG...STSTRYLMKDLSPWN...LEKGIIVYI 415  
paTex ...TLAVLAALCAKHQVELIAIGNGTASRETDKLAGELIKKYPGMR...LTKIMVS 420

### YqgF

hsSpt6 ELVDNELAILVMNSKKSEAEAFRDYPPVLRQAVSLARRIQDPLIEFAQVCCSSDEDIICLKLF 942  
scSpt6 IYVEDEVAIRVONSERAAAEFFNKPPLVKYCTALARHMHSPLEVANL...TSEVRSLSI 904  
ecSpt6 ...P.VVYVESRAHTKAED...YFCCNIAIRIQCPLEYS...LEKGIIVYI 460  
paTex EAGASVYSASELAAKEFPELDDVSLRGAVSIARRLODPLAEVLKTI...EPKSIIGVQOYO 475

hsSpt6 HPLQEHVYKEELNLALYCEFVTRVNEVGVVNRARIAHPYSQALLIYVCGLGPRKGGHLLK 1002  
scSpt6 HPHQNLLESSEQLSWALGETAFVDVNLVVEVNAKATDNNDYASAKYISGFGKRRKAIDFLLQ 964  
ecSpt6 QQ...QILPRNVMEVETIRKGIILTAISVGVVNVLLNRRRKEGLSLIPTSSSLKSS... 514  
paTex HDVSOIKLARSLDAVVEDC...VNAVGVVDVNTA.SAALLARISGLNSTLAQNIVA... 526

### HhH

hsSpt6 IILKQNNTRESRTOQLVTMCHMGPKVFMMNCAGFLKID.TASLGDSDTDSYTEELDGSRVVHPE 1061  
scSpt6 SLQRLNNEPLARQQOLITHNILHKTIFMNSAGFLYISWNEKRRQYEDLEHQLDSTRIHVEK 1024  
ecSpt6 .LSDLRVMKLEDKDLCE.SEVEYDNLVTYIRVHPVLL...SRVSGSILDAATVHPE 568  
paTex .HRDANGAFRTRDEKKSRSRLGEKTFEQAGFLRVH...NGDNPLDASRVVHPE 575

hsSpt6 IYEWARKMAVDALEYDES.AEEDANPAGAEELLE...N...PERLKDLDLDAFAEELE 1113  
scSpt6 VYHLATKVAAADALEYDPTIAKKEEQGTMEFELELREDPPDRRAKLESNLSYAEEL 1084  
ecSpt6 NYDVVRKKTCTDLCEEG...VDKNLDPVKALSN...A...KELKTSRPKILEC...G... 616  
paTex T.VLVRQRIADT...ERDTRSLIG...D...SAFLKRLDPFKKFTDET... 613

hsSpt6 QYGGDKHITLYDIRAELSCRYKDLRTAYRSPNTEEIFNMILTKETPETEYI...GLIICNVITG 1173  
scSpt6 NTGLRKLNNNTIIVLDELNDFFLQGGDLEIFQSLTIGSEKTFEFGLIPVVEK 1144  
ecSpt6 ...DPLTTIDVYKLV...GDRSIFGLPDDHVYKELTIG...CDESLLGKVVVVEGGLISK 865  
paTex .FG...LPVTDILKELDKPGRDPPEFKFAEFQEVESL...KDLKEGMVLEGVVITN 664

hsSpt6 IAHRRRPQGESYDQAIRNDETGLWQCFFCQQDHFPELSEVWNHFDGSGCPCQAIGVKTRLD 1233  
scSpt6 FWHN...L...DIICTTN 1155  
ecSpt6 CGNT...YYLLDSN 676  
paTex VTNF...GAFVDIG 675

### S1

hsSpt6 NGVTGELPTKFLSDKVVKRPEERV.KVGMTVHCRIMKIDIEKFSADLTLCRTSGLMDRNE 1292  
scSpt6 SEVECVVNAAQRHAGACLRRPANEIYIEIGKTYPAKVYIDYANITAEVSLDHDVVKQYV 1215  
ecSpt6 ATPAAIYIRKNRGQQLF...LNQLVTVRIEYMNDFLLSYTASIAVSEQKKNHS 728  
paTex VHQDGLVHISALSSEKPKDPEYV.V.KAGDITKVKVMEVDIPRRNVGLSMRMSDTPGKEV 734

hsSpt6 WKLPKD...TYDYFDAEADAHKQEEDMKRQOORTTYIKRVIAPHSFHNIINFKQAEKMMETM 1350  
scSpt6 .ISYSKDPHIWDLKQLEDAEERKLMMAEAKRTRHVRVNHVYFFPNGRQAEDYTRSK 1274  
ecSpt6 ...RFMIHPLFKDNLNSEQSEYELKDH 751  
paTex GQRGRPAGSGQPRQERGAIRGQSQAPPANNAMEALFANAQQLKKK\* 779

hsSpt6 DQGDVITRRSSKGENHLTVITWKVSDGYVQHYDVREEGKENAFSLGATLWINSEEFEOLD 1410  
scSpt6 ERGEFVIRGSSRRGDDHLVITWKDKDLFQHIIDIEEKENFLAIGKVLVDNQKVNDDLDQ 1334  
ecSpt6 .SSSILLRSMRCKDCSP.VVVLKVSDBGVYHMKLIEAEAKKY...GNS...YOLD 800  
paTex

### Tandem SH2

hsSpt6 IIVARYVQPMASFARDLNHKYVQDCSGGDRKKLEELIKTKKEKPTFIPVFICACKEIPG 1470  
scSpt6 IIVEYLQNKVRLLENTSSEKFKS...GTKKDV.VKVIEDYSRVNPNKSVVVFSLNHDPG 1391  
ecSpt6 FLSRVAKMMLTNFKNKNHKYVFE...NEEKLLDYLNOGGSYIRVGFCLSRKYPG 852  
paTex

hsSpt6 KFLLGIVQ...DRGKPRIEYVITVPEGFRYRGOIFPTVINGLFRWFKDHYPQVVPGITPSSSS 1528  
scSpt6 WFLMLFKINANSKLTYTNVVKLTINTGYFLVNYNYPVSIQLCNGFKTLLKSNSSKNRMNYR\*1451 1451  
ecSpt6 KLCMLYR...DGRNHKEYITVD.EHLVYEGRNFAVSLDEFVRYRKSL\* 894  
paTex

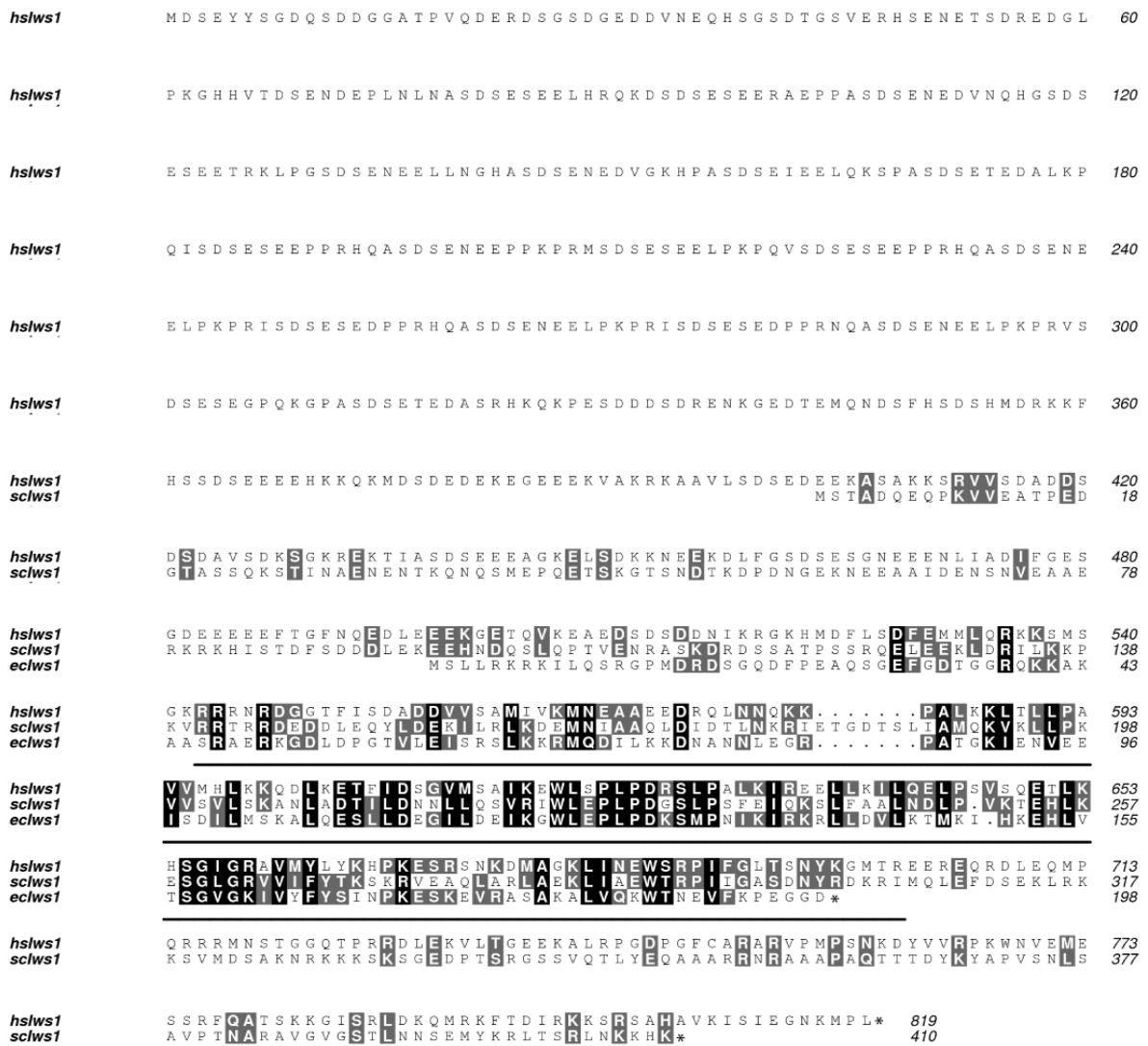
hsSpt6 RTR.TPASINATPANINLADLTRAVALPQNMTSQMFSIAIAAVTGQGQNPATPAQWASS 1587  
scSpt6  
ecSpt6  
paTex

hsSpt6 QYGYGGSGSSAYHVFPPTAQQPVATPLMTPSYSYITTPSQPITTPQYHQLQASTTPQSA 1647  
scSpt6  
ecSpt6  
paTex

hsSpt6 QAQPQPSSSRQRQQPKSNSHAAIDWGKMAEQWLQKEAERRKQKQRLTRPSPSPMIE 1707  
scSpt6  
ecSpt6  
paTex

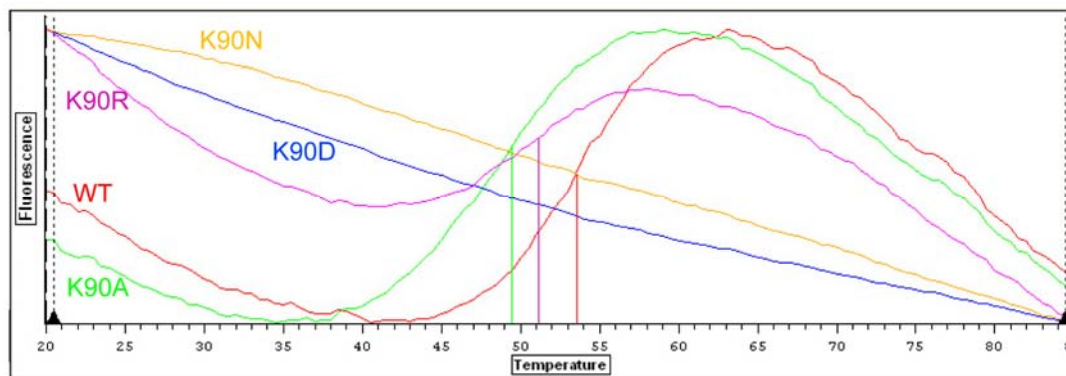
hsSpt6 STPMSIAGDATPLLDEMDR\* 1726  
scSpt6  
ecSpt6  
paTex

B

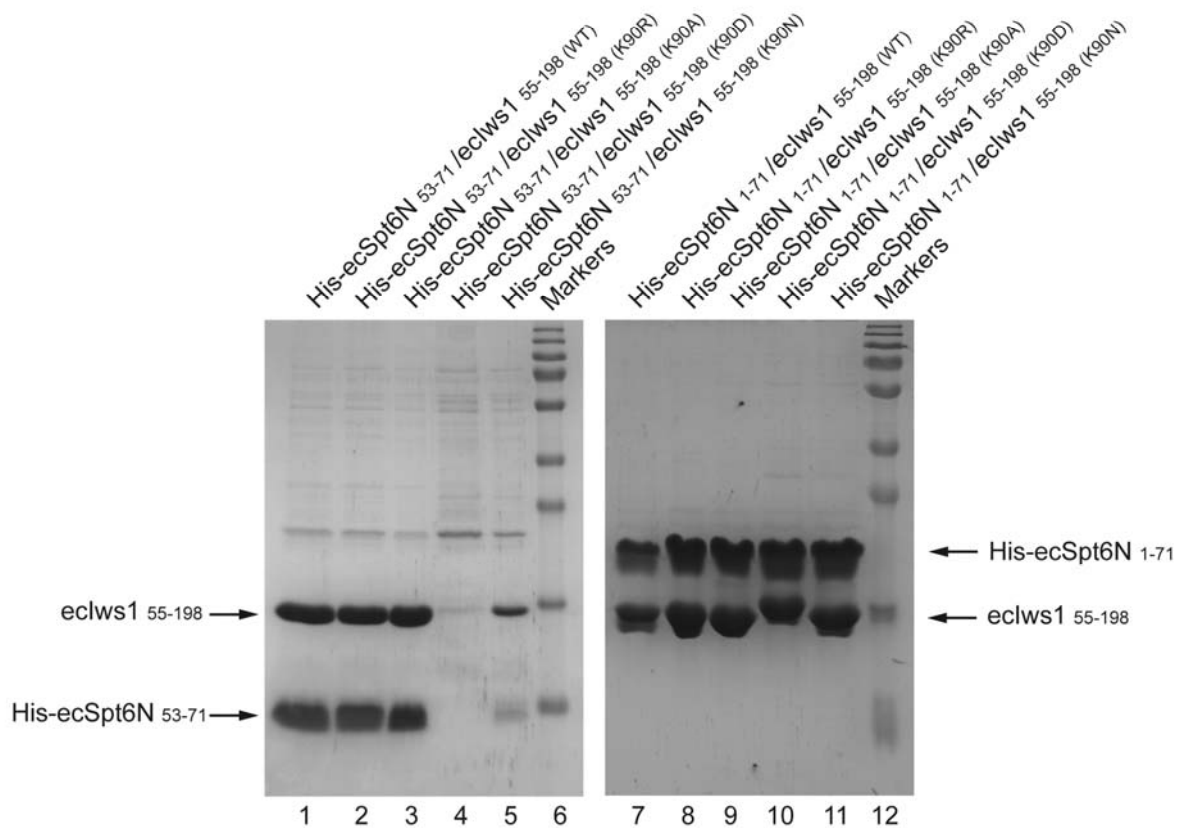


**Supplementary Figure 1.** Multiple alignments of full-length Spt6, Tex and Iws1 proteins. *ec*, *Encephalitozoon cuniculi*; *sc*, *Saccharomyces cerevisiae*; *hs*, *Homo sapiens*; *pa*, *Pseudomonas aeruginosa*. Sequence similarities are indicated by shading. End of sequences are marked with a “\*”. (A) Multiple sequence alignment of *ec*, *sc*, *hs* Spt6 and Spt6 bacterial paralog *pa*Tex. The specific conserved region at the N-terminus of Spt6 (Spt6N) is indicated with a red box. The structural domains of Tex as well as the Spt6-specific tandem SH2 domains are indicated underneath the sequences. (B) Multiple sequence alignment of *ec*, *sc*, *hs* Iws1. The region sufficient for the essential function of Iws1 in yeast is underlined.

A

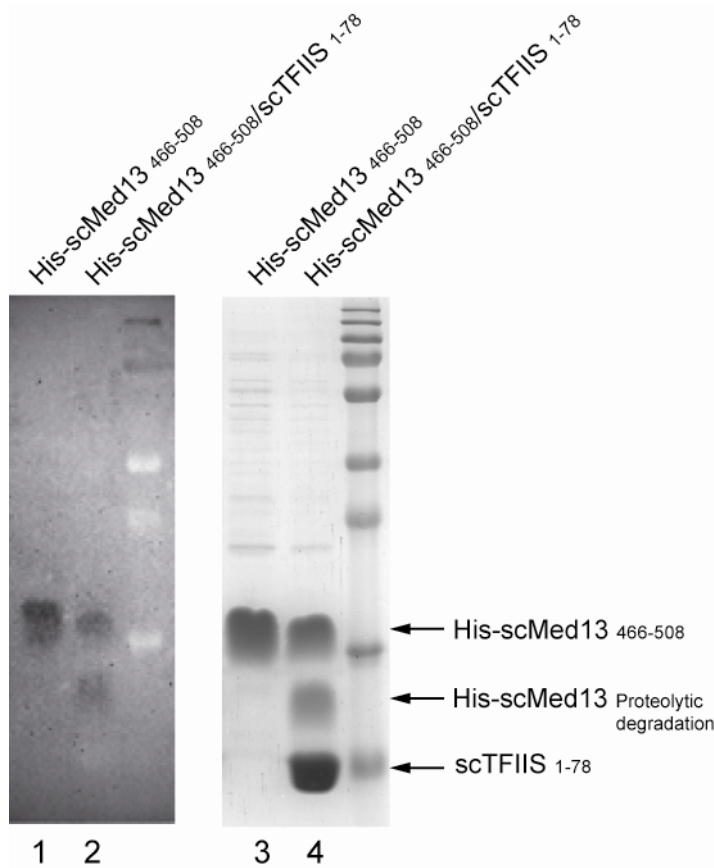


B



Supplementary Figure 2. Effect of K90 mutants on *E. cucurbitur* Iws1 stability and Spt6N binding.

(A) Biophysical characterization of *E. cucurbitur* Iws1 wild-type (WT) and K90 mutants using the Thermofluor technology. For each protein, the fluorescence observed upon binding of the SYPRO orange dye to the protein is plotted versus the temperature. (B) Effect of K90 mutations on formation of the complexes between *ecIws1*<sup>55-198</sup> and *ecSpt6N*<sup>53-71</sup> or *ecSpt6N*<sup>1-71</sup>. The K90D mutant migrates more slowly (lane 10).



Supplementary Figure 3. A C-terminal degraded fragment of *scMed13* is solubilised upon binding to TFIIS.

Upon co-expression of His-*scMed13*<sub>466-508</sub> with *scTFIIS*<sub>1-78</sub>, a small protein is observed, bound to the affinity resin, which does not migrate at the expected molecular weight of His-*scMed13*<sub>466-508</sub> and *scTFIIS*<sub>1-78</sub> (right panel; Coomassie-stained SDS gel). A western blot analysis (left panel) using an anti-polyhistidine antibody shows that this protein is his-tagged and corresponds most likely to a C-terminal degradation of His-*scMed13*<sub>466-508</sub> which is solubilised through its interaction with *scTFIIS*<sub>1-78</sub>.

Supplementary Table I. Data collection and refinement statistics for *E. cuniculi* Iws1<sub>55-198</sub>

<b>Data collection statistics</b>				
<i>Data sets</i>	Native	Se-Met $\lambda 1$ peak	Se-Met $\lambda 2$ inflexion	Se-Met $\lambda 3$ remote
<i>Wavelength (Å)</i>	0.933	0.9792	0.9795	0.9753
<i>Space group</i>	P2 <sub>1</sub>	P2 <sub>1</sub>	P2 <sub>1</sub>	P2 <sub>1</sub>
<b>Cell constants</b>				
<i>a (Å)</i>	33.7	41.3	41.4	41.3
<i>b (Å)</i>	128.8	218.3	218.5	218.6
<i>c (Å)</i>	33.6	71.7	72.0	72.0
<i>β (°)</i>	101.2	102.7	102.7	102.8
<i>Resolution (Å)</i>	64-2.25	30-2.7	30-3.0	30-3.0
<i>Reflections (measured/unique)</i>	60417 13209	245211 68237	167714 49682	136789 47418
<i>Redundancy (overall/last shell)</i>	4.6/4.4	3.6/3.6	3.4/3.4	2.9/2.9
<i>Completeness (%) (overall/last shell)</i>	99.2/99.3	99.7/99.9	99.7/100	98.4/100
<i>R<sub>sym</sub><sup>S</sup> (%) (overall/last shell)</i>	5.9/12.3	7.5/18.2	7.3/19.1	8.1/21.0
<i>I/σ(I) (overall/last shell)</i>	25.9/15.1	17.9/7.6	16.9/6.2	15.8/6.4
<b>Refinement statistics</b>				
<i>Resolution (Å)</i>	64-2.25			
<i>Number of protein atoms</i>	2268			
<i>Number of water molecules/ions</i>	35			
<i>Number of reflections (F&gt;0)</i> <i>(all/test set)</i>	12534 650			
<i>R-factor<sup>#</sup> (%)</i>	20.4			
<i>R-free<sup>#</sup> (%)</i>	26.5			
<b>Deviations from ideal geometry</b>				
<i>Bonds (Å)</i>	0.019			
<i>Angles (°)</i>	1.780			
<i>Mean temperature factors (Å<sup>2</sup>)</i>	15.79			

<sup>S</sup>  $R_{sym} = \sum |I - \langle I \rangle| / \sum I$ , where  $I$  is the observed intensity and  $\langle I \rangle$  is the average intensity for multiple measurements.

<sup>#</sup>  $R = \sum_{hkl} |F_{obs} - F_{calc}| / \sum_{hkl} F_{obs}$ , where  $F_{obs}$  and  $F_{calc}$  are the observed and calculated structure factor amplitudes for reflection  $hkl$ , applied to the work (R-factor) and test (R-free) sets.

Supplementary Table II. Data collection and refinement statistics for *E. cuniculi* Iws1<sub>55-198</sub>/Spt6N complexes

<b>Data collection statistics</b>			
<i>Data sets</i>	ecSpt6N <sub>53-71</sub> ecIws1 <sub>55-198</sub> Form1	ecSpt6N <sub>53-71</sub> ecIws1 <sub>55-198</sub> Form2	ecSpt6N <sub>34-71</sub> ecIws1 <sub>55-198</sub>
<i>Wavelength (Å)</i>	0.9762	0.9334	0.977
<i>Space group</i>	P4 <sub>1</sub> 2 <sub>1</sub> 2	P6 <sub>5</sub>	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
<b>Cell constants</b>			
<i>a (Å)</i>	52.3	112.6	27.4
<i>b (Å)</i>	52.3	112.6	56.1
<i>c (Å)</i>	151.1	51.4	116.4
<i>β (°)</i>	90	120	90
<i>Resolution (Å)</i>	50-1.95	100-2.1	58-1.75
<i>Reflections (measured/unique)</i>	199644 16077	234782 21935	85689 19008
<i>Redundancy (overall/last shell)</i>	12.4/11.4	10.7/10.2	4.5/4.6
<i>Completeness (%) (overall/last shell)</i>	99.4/99.6	99.9/100	99.4/99.9
<i>R<sub>sym</sub><sup>s</sup> (%) (overall/last shell)</i>	4.1/17.1	8.5/28.8	4.2/16.6
<i>I/σ(I) (overall/last shell)</i>	78.8/17.0	33.6/10.5	44.6/9.2
<b>Refinement statistics</b>			
<i>Resolution (Å)</i>	50-1.95	100-2.1	58-1.75
<i>Number of protein atoms</i>	1269	2474	1300
<i>Number of water molecules/ions</i>	133	153	170
<i>Number of reflections (F&gt;0)</i> <i>(all/test set)</i>	15204 803	20780 1124	17980 973
<i>R-factor<sup>#</sup> (%)</i>	21.0	20.0	19.4
<i>R-free<sup>#</sup> (%)</i>	23.6	24.7	23.6
<b>Deviations from ideal geometry</b>			
<i>Bonds (Å)</i>	0.017	0.017	0.018
<i>Angles (°)</i>	1.49	1.57	1.61
<i>Mean temperature factors (Å<sup>2</sup>)</i>	16.15	11.63	11.89

<sup>s</sup>  $R_{sym} = \sum |I - \langle I \rangle| / \sum I$ , where *I* is the observed intensity and  $\langle I \rangle$  is the average intensity for multiple measurements.

<sup>#</sup>  $R = \sum_{hkl} |F_{obs} - F_{calc}| / \sum_{hkl} F_{obs}$ , where *F<sub>obs</sub>* and *F<sub>calc</sub>* are the observed and calculated structure factor amplitudes for reflection *hkl*, applied to the work (R-factor) and test (R-free) sets.

Supplementary Table III. Yeast strains.

<b>Strain</b>	<b>Genotype</b>
FY2801	<i>MAT<math>\alpha</math> his4-912<math>\delta</math> lys2-128<math>\delta</math> leu2<math>\Delta</math>1 ura3-52 trp1<math>\Delta</math>63 spt6<math>\Delta</math>::LEU2 (CEN, TRP1, FLAG-SPT6)</i>
FY2802	<i>MAT<math>\alpha</math> his4-912<math>\delta</math> lys2-128<math>\delta</math> leu2<math>\Delta</math>1 ura3-52 trp1<math>\Delta</math>63 spt6<math>\Delta</math>::LEU2 (CEN, TRP1, spt6-50)</i>
FY2803	<i>MAT<math>\alpha</math> his4-912<math>\delta</math> lys2-128<math>\delta</math> leu2<math>\Delta</math>1 ura3-52 trp1<math>\Delta</math>63 spt6<math>\Delta</math>::LEU2 (CEN, TRP1, FLAG-spt6-Y255A,W257A)</i>
FY2804	<i>MAT<math>\alpha</math> his4-912<math>\delta</math> lys2-128<math>\delta</math> leu2<math>\Delta</math>1 ura3-52 trp1<math>\Delta</math>63 spt6<math>\Delta</math>::LEU2 (CEN, TRP1, FLAG0spt6-Y255A)</i>
FY2805	<i>MAT<math>\alpha</math> his4-912<math>\delta</math> lys2-128<math>\delta</math> leu2<math>\Delta</math>1 ura3-52 trp1<math>\Delta</math>63 spt6<math>\Delta</math>::LEU2 (CEN, TRP1,FLAG- spt6-W257A)</i>
FY2806	<i>MAT<math>\alpha</math> his4-912<math>\delta</math> lys2-128<math>\delta</math> leu2<math>\Delta</math>1 ura3-52 trp1<math>\Delta</math>63 spt6<math>\Delta</math>::LEU2 (CEN, TRP1, FLAG-spt6-I248A,F249A)</i>
FY2807	<i>MAT<math>\alpha</math> his4-912<math>\delta</math> lys2-128<math>\delta</math> leu2<math>\Delta</math>1 ura3-52 trp1<math>\Delta</math>63 spt6<math>\Delta</math>::LEU2 (CEN, TRP1, FLAG-spt6-G250A,G252A)</i>