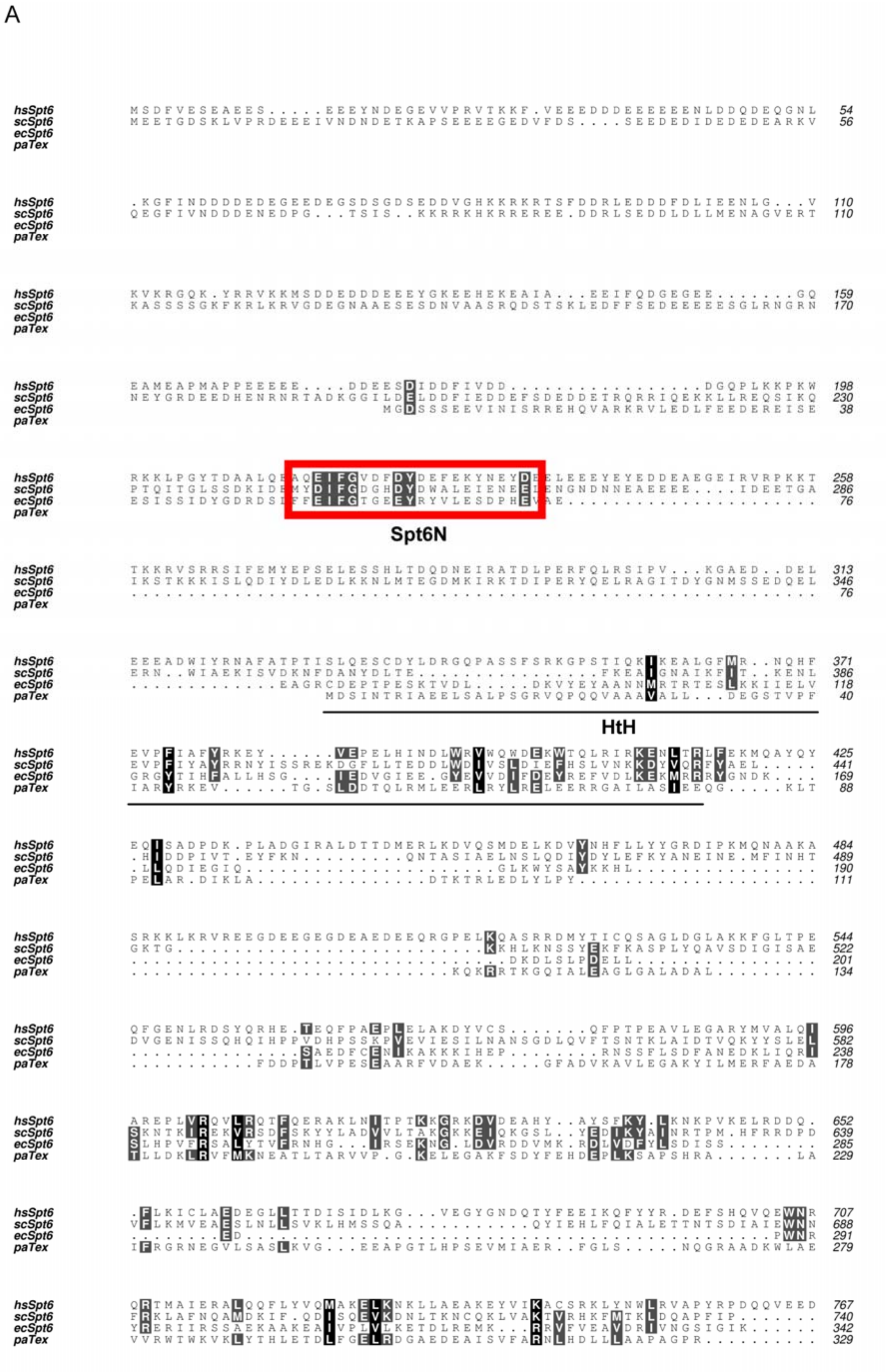
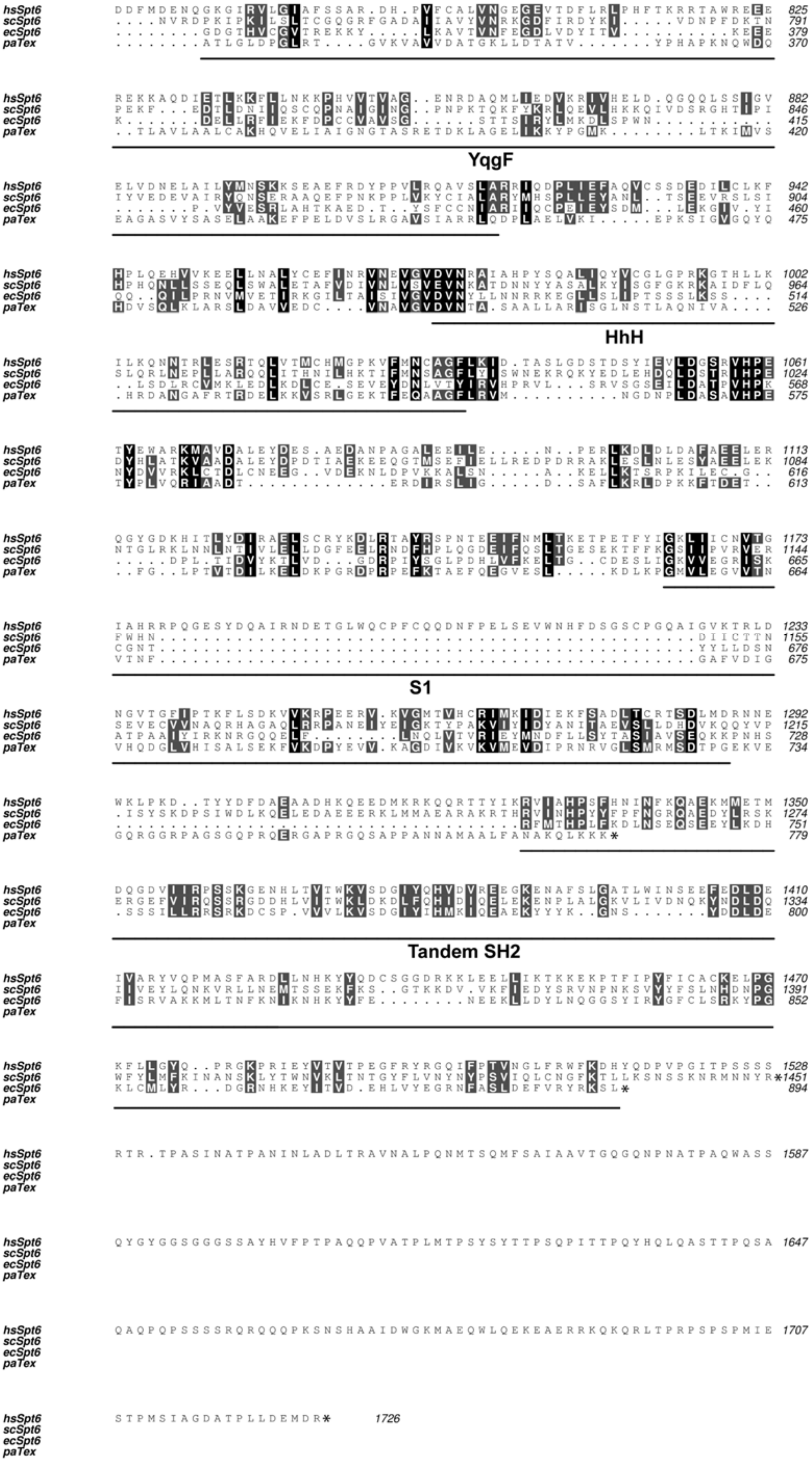
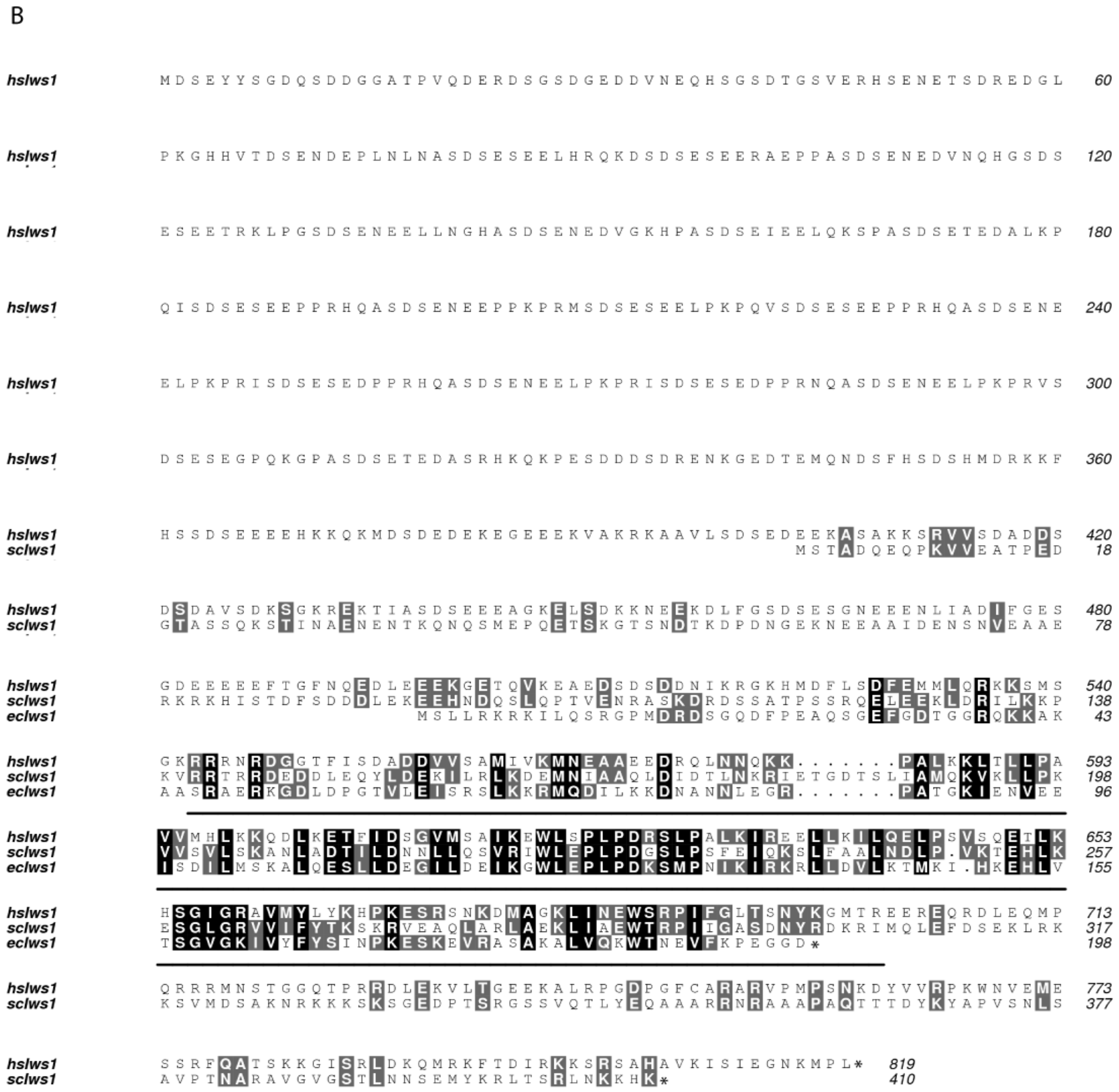
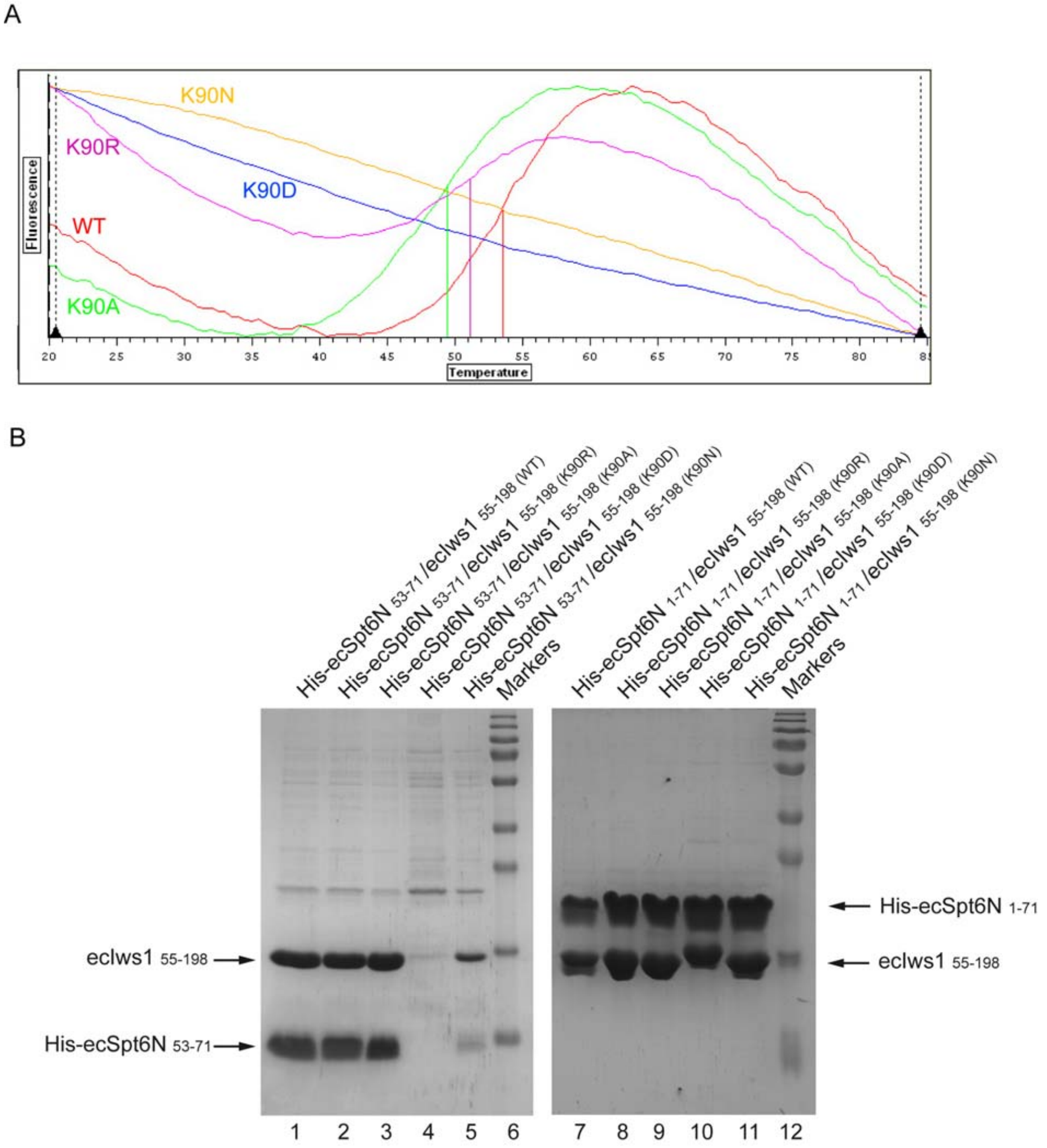

A
hsSpt6
scSpt6
ecSpt6
paTex
Spt6N
HtH


hsSpt6
scSpt6
ecSpt6
paTex
YqgF
HhH
S1
Tandem SH2
825
791
379
370
882
846
415
420
942
904
460
475
1002
964
514
526
1061
1024
568
575
1113
1084
616
613
1173
1144
665
664
1233
1155
676
675
1292
1215
728
734
1350
1274
751
779
1410
1334
800
1470
1391
852
1528
1451
894
1587
1647
1707
1726

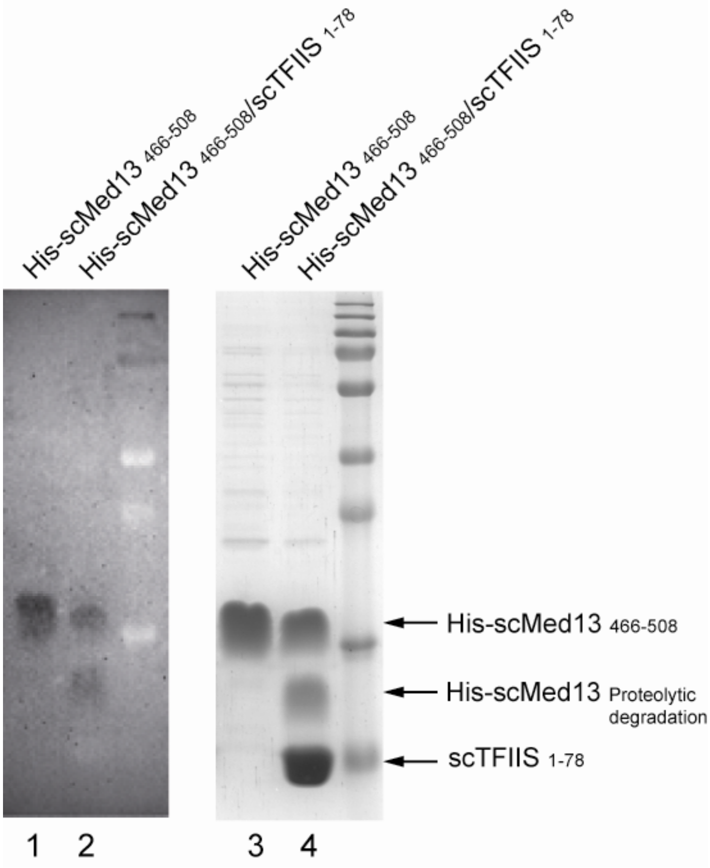


Supplementary Figure 1. Multiple alignments of full-length Spt6, Tex and Iws1 proteins. *ec*, *Encephalitozoon cuniculi*; *sc*, *Saccharomyces cerevisiae*; *hs*, *Homo sapiens*; *pa*, *Pseudomonas aeruginosa*. Sequence similarities are indicated by shading. End of sequences are marked with a '*'. (A) 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.



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

(A) Biophysical characterization of *E. cuniculi* 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 *ec*Iws1$_{55\text{-}198}$ and *ec*Spt6N$_{53\text{-}71}$ or *ec*Spt6N$_{1\text{-}71}$. The K90D mutant migrates more slowly (lane 10).



Supplementary Figure 3. A C-terminal degraded fragment of *sc*Med13 is solubilised upon binding to TFIIS.

Upon co-expression of His-*sc*Med13$_{466\text{-}508}$ with *sc*TFIIS$_{1\text{-}78}$, a small protein is observed, bound to the affinity resin, which does not migrate at the expected molecular weight of His-*sc*Med13$_{466\text{-}508}$ and *sc*TFIIS$_{1\text{-}78}$ (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-*sc*Med13$_{466\text{-}508}$ which is solubilised through its interaction with *sc*TFIIS$_{1\text{-}78}$.

Supplementary Table I. Data collection and refinement statistics for *E. cuniculi* Iws1$_{55-198}$

Data collection statistics				
Data sets	Native	Se-Met λ1 peak	Se-Met λ2 inflexion	Se-Met λ3 remote
Wavelength (Å)	0.933	0.9792	0.9795	0.9753
Space group	P2$_1$	P2$_1$	P2$_1$	P2$_1$
Cell constants				
a (Å)	33.7	41.3	41.4	41.3
b (Å)	128.8	218.3	218.5	218.6
c (Å)	33.6	71.7	72.0	72.0
β (°)	101.2	102.7	102.7	102.8
Resolution (Å)	64-2.25	30-2.7	30-3.0	30-3.0
Reflections (measured/unique)	60417 13209	245211 68237	167714 49682	136789 47418
Redundancy (overall/last shell)	4.6/4.4	3.6/3.6	3.4/3.4	2.9/2.9
Completeness (%) (overall/last shell)	99.2/99.3	99.7/99.9	99.7/100	98.4/100
$R_{sym}^{\$}$ (%) (overall/last shell)	5.9/12.3	7.5/18.2	7.3/19.1	8.1/21.0
$I/\sigma(I)$ (overall/last shell)	25.9/15.1	17.9/7.6	16.9/6.2	15.8/6.4
Refinement statistics				
Resolution (Å)	64-2.25			
Number of protein atoms	2268			
Number of water molecules/ions	35			
Number of reflections (F>0) (all/test set)	12534 650			
R-factor$^{\#}$ (%)	20.4			
R-free$^{\#}$ (%)	26.5			
Deviations from ideal geometry				
Bonds (Å)	0.019			
Angles (°)	1.780			
Mean temperature factors ($Å^2$)	15.79			

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

[#] $R = \Sigma_{hkl}|F_{obs} - F_{calc}|/\Sigma_{hkl}F_{obs}$, 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$_{55-198}$/Spt6N complexes

Data collection statistics			
Data sets	ecSpt6N$_{53-71}$ ecIws1$_{55-198}$ Form1	ecSpt6N$_{53-71}$ ecIws1$_{55-198}$ Form2	ecSpt6N$_{34-71}$ ecIws1$_{55-198}$
Wavelength (Å)	0.9762	0.9334	0.977
Space group	$P4_12_12$	$P6_5$	$P2_12_12_1$
Cell constants			
a (Å)	52.3	112.6	27.4
b (Å)	52.3	112.6	56.1
c (Å)	151.1	51.4	116.4
β (°)	90	120	90
Resolution (Å)	50-1.95	100-2.1	58-1.75
Reflections (measured/unique)	199644 16077	234782 21935	85689 19008
Redundancy (overall/last shell)	12.4/11.4	10.7/10.2	4.5/4.6
Completeness (%) (overall/last shell)	99.4/99.6	99.9/100	99.4/99.9
R_{sym}[$] (%) (overall/last shell)	4.1/17.1	8.5/28.8	4.2/16.6
$I/\sigma(I)$ (overall/last shell)	78.8/17.0	33.6/10.5	44.6/9.2
Refinement statistics			
Resolution (Å)	50-1.95	100-2.1	58-1.75
Number of protein atoms	1269	2474	1300
Number of water molecules/ions	133	153	170
Number of reflections (F>0) (all/test set)	15204 803	20780 1124	17980 973
R-factor[#] (%)	21.0	20.0	19.4
R-free[#] (%)	23.6	24.7	23.6
Deviations from ideal geometry			
Bonds (Å)	0.017	0.017	0.018
Angles (°)	1.49	1.57	1.61
Mean temperature factors ($Å^2$)	16.15	11.63	11.89

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

[#] $R = \Sigma_{hkl}|F_{obs} - F_{calc}|/\Sigma_{hkl}F_{obs}$, 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 III. Yeast strains.

Strain	**Genotype**
FY2801	*MATα his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt6Δ::LEU2 (CEN, TRP1, FLAG-SPT6)*
FY2802	*MATα his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt6Δ::LEU2 (CEN, TRP1, spt6-50)*
FY2803	*MATα his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt6Δ::LEU2 (CEN, TRP1, FLAG-spt6-Y255A,W257A)*
FY2804	*MATα his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt6Δ::LEU2 (CEN, TRP1, FLAG0spt6-Y255A)*
FY2805	*MATα his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt6Δ::LEU2 (CEN, TRP1,FLAG- spt6-W257A)*
FY2806	*MATα his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt6Δ::LEU2 (CEN, TRP1, FLAG-spt6-I248A,F249A)*
FY2807	*MATα his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt6Δ::LEU2 (CEN, TRP1, FLAG-spt6-G250A,G252A)*