Supporting information

The spliceosomal PRP19 complex of trypanosomes

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Content

Table S1. List of oligonucleotides

Purpose	Description	Sequence
RT-PCR	*SPF27* mRNA	5′-AAGCTTACGCGTCCCATGGTGCTTCCTTAC-3′ 5′-TCTAGACCCGAACGTTTCTCAGCC-3′
RT-PCR	7SL RNA	5′-TTGCTCTGTAACCTTC-3′ 5′-TCTACAGTGGCGACCTCAAC-3′
RT-PCR	*ORC1* mRNA	5′-AAGCTTACGCGTCGAAGGAGGGACAGTAAC-3′ 5′-TCTAGAAAAACGCATACGGTAGAC-3′
RT-[q]PCR	α tubulin pre-mRNA	5′-GTAAGTGGTGGTGGCGTAAG-3′ 5′-CAATGTGGATGCAGATAGCC-3′
RT-[q]PCR	α tubulin mRNA	5′-ACAGTTTCTGATCTATATTGATCTT-3′ 5′-GAGAGTTGCTCGTGGTAGGC-3′
Competitive RT-PCR	*PAP* [pre-]mRNA	5′-GTGCAGCGGCACTCCCAAAAC-3′ 5′-CGTTAAAACAGATGGACAAATC-3′
RT-qPCR	SL RNA	5′-ACAGTTTCTGTACTATATTG-3′ 5′-CGACCCCACCTTCCAGATTC-3′
RT-qPCR	*RPB7* pre-mRNA	5′-CCACTCGAAGGAGTAGTTTTC-3′ 5′-TTATGTGCACTTGCTGGTG-3′
RT-qPCR	*RPB7* mRNA	5′-CATGGGCCCGAGAGGAATATAAAAGTGGAGCCTC-3′ 5′-ATTCTGATTTGTGCGGGC-3′
RT-qPCR	*PAP* pre-mRNA (exon 1-intron)	5′-GTGCAGCGGCACTCCCAAAAC-3′ 5′-GGGATTAAGGAAAGAACTCAC-3′
RT-qPCR	18S rRNA	5′-TCATCAAACTGTGCCGATTAC-3′ 5′-CTATTGAAGCAATATCGG-3′
Primer extension	SL_PE (SL RNA) SL40 (SL RNA) 7SL_PE (7SL RNA) U1_PE (U1 snRNA) U2f (U2 snRNA) U4_PE (U4 snRNA) U5_PE (U5 snRNA) U6_PE (U6 snRNA)	5′-CGACCCCACCTTCCAGATTC-3′ 5′-CTACTGGGAGCTTCTCATAC-3′ 5′-GAACCCCCGCTTGTC-3′ 5′-AGCACGGCGCTTTCGTGATG-3′ 5′-ACAGGCAACAGTTTTGATCC-3′ 5′-TACCGGATATAGTATTGCAC-3′ 5′-GGGAGAGTGCTAATCTTCTC-3′ 5′-GAACCCCCGCTTGTC-3′

The gene-specific sequence is underlined when oligonucleotides carry additional nucleotides.

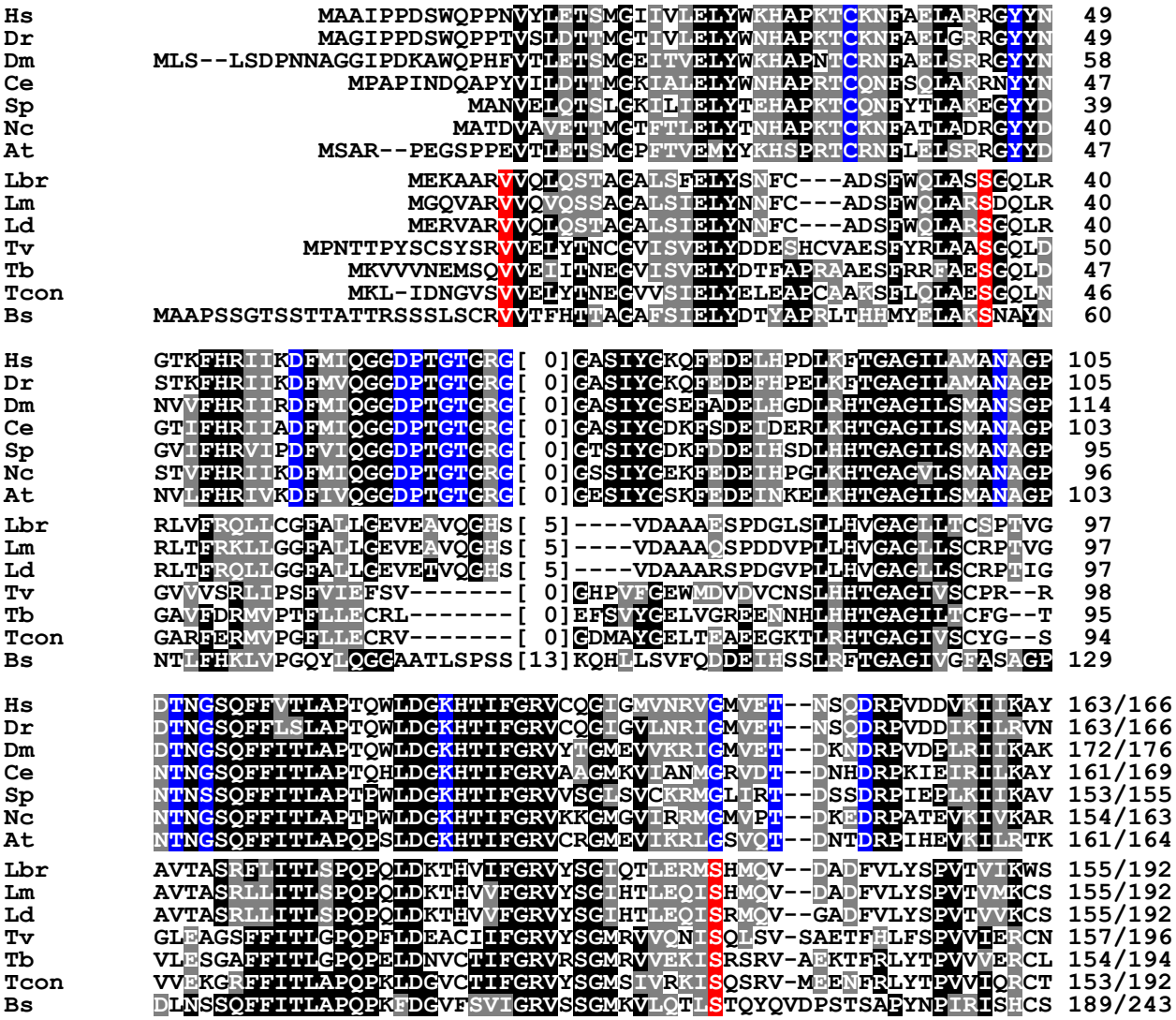

Fig. S1. Multiple sequence alignment of PPIL1 orthologues.

Clustal Omega (McWilliam *et al.*, 2013) was used at default parameters to align PPIL1 amino acid sequences from *Homo sapiens* (Hs, accession number NP_057143), *Danio rerio (Dr,* NP_001029350), *Drosophila melanogaster* (Dm, NP_523874), *Caenorhabditis elegans* (Ce, NP_501118), *Schizosaccharomyces pombe* (Sp, NP_593308), *Neurospora crassa* (Nc, XP_964739), *Arabidopsis thaliana* (At, NP_181157), and from the kinetoplastid species *Leishmania braziliensis* (Lbr, LbrM.23.0140), *Leishmania major* (Lm, LmjF.23.0125), *Leishmania donovani* (Ld, LdBPK_230140.1), *Trypanosoma vivax* (Tv, TvY486_0801580), *Trypanosoma brucei* (Tb, Tb927.8.2090), *Trypanosoma congolense* (Tc, TcIL3000_8_2090), and *Bodo saltans* (Bs, BS78785.1..pep). Positions with more than 50% similarity or identity are shaded in gray and black, respectively. Identical positions in model organisms without conservation in kinetoplastids are shaded blue and identical position is kinetoplastids without conservation in model organisms are shaded in red. A hyphen indicates lack of an amino acid at this position. Numbers in parentheses specify lengths of non-conserved insertions. *Saccharomyces cerevisiae* and *Trypanosoma cruzi* appear to lack a PPIL1 homolog.

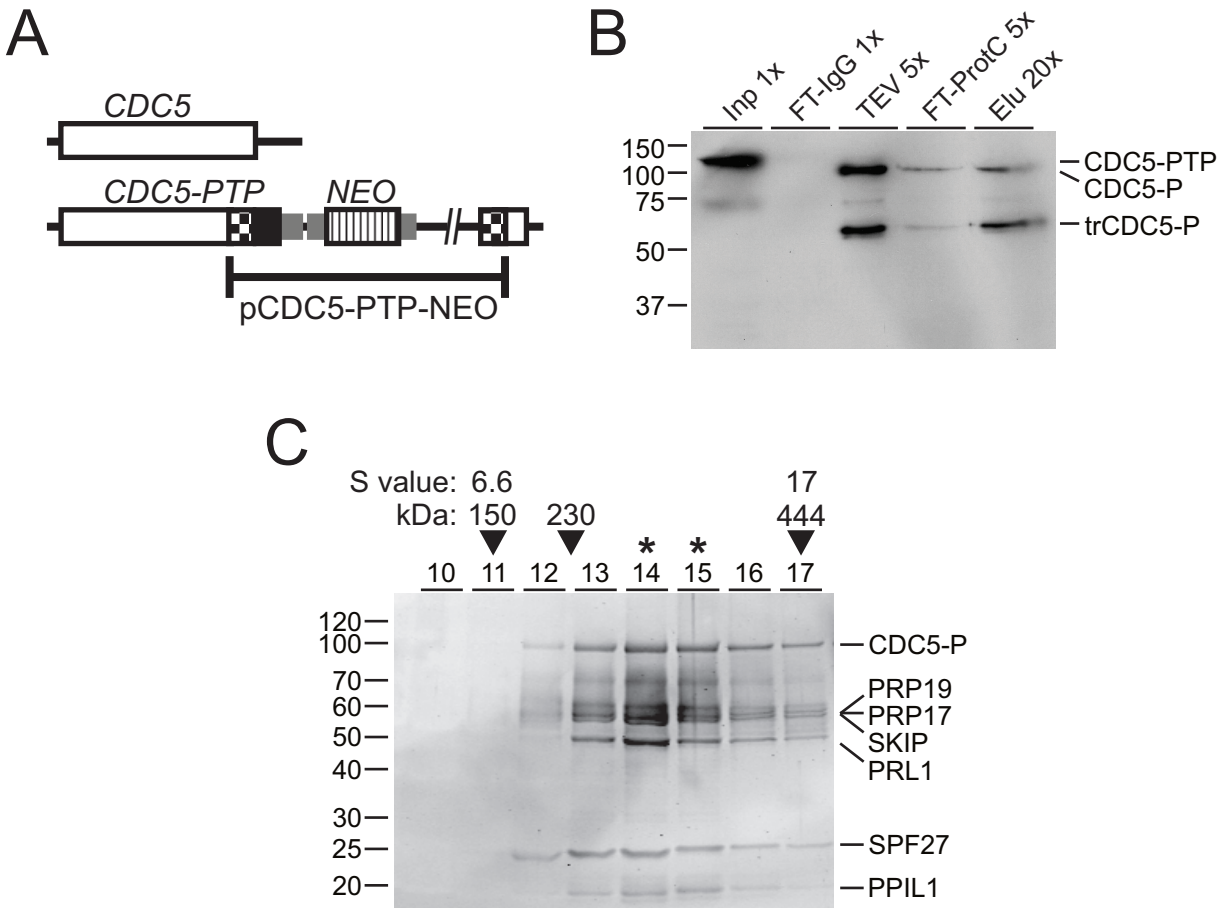

Fig. S2. PTP tagging and tandem affinity purification of CDC5 isolated the complete PRP19 complex

A. Schematic (not to scale) of the *CDC5* locus after integration of pCDC5-PTP-NEO, fusing the PTP coding sequence (black box) to an endogenous allele.

B. Immunoblot monitoring of the CDC5-PTP tandem affinity purification CDC5-PTP and CDC5-P (after removal of the ProtA domains) were detected with the anti-ProtC HPC4 antibody in crude extract (Inp), flowthrough of the IgG column (FT-IgG), TEV protease eluate (TEV), flowthrough of the anti-ProtC column (FT-ProtC) and the final eluate (Elu). x-Values indicate relative amounts loaded. Apparently, during extract preparation and purification, part of CDC5 was converted into a truncated form in which an N-terminal domain was cleaved off (trCDC5).

C. The final eluate of a CDC5-PTP tandem affinity purification was sedimented through a linear sucrose gradient, fractionated, and visualized by Sypro Ruby staining of an SDS-PAGE gel, the same as with PRP19 shown in figure 1D. Protein bands were excised and protein identities confirmed by LC/MS/MS. Asterisks indicate the sedimentation peak.

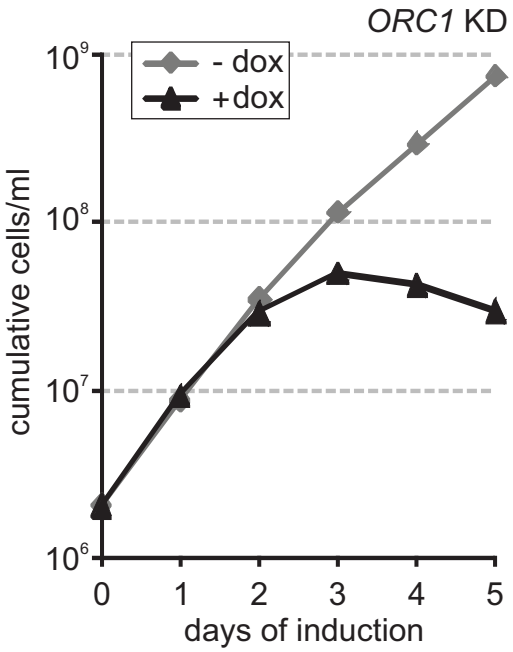

Fig. S3. *ORC1* silencing

Procyclic trypanosome culture growth in the absence and presence of doxycycline which induced *ORC1* silencing.

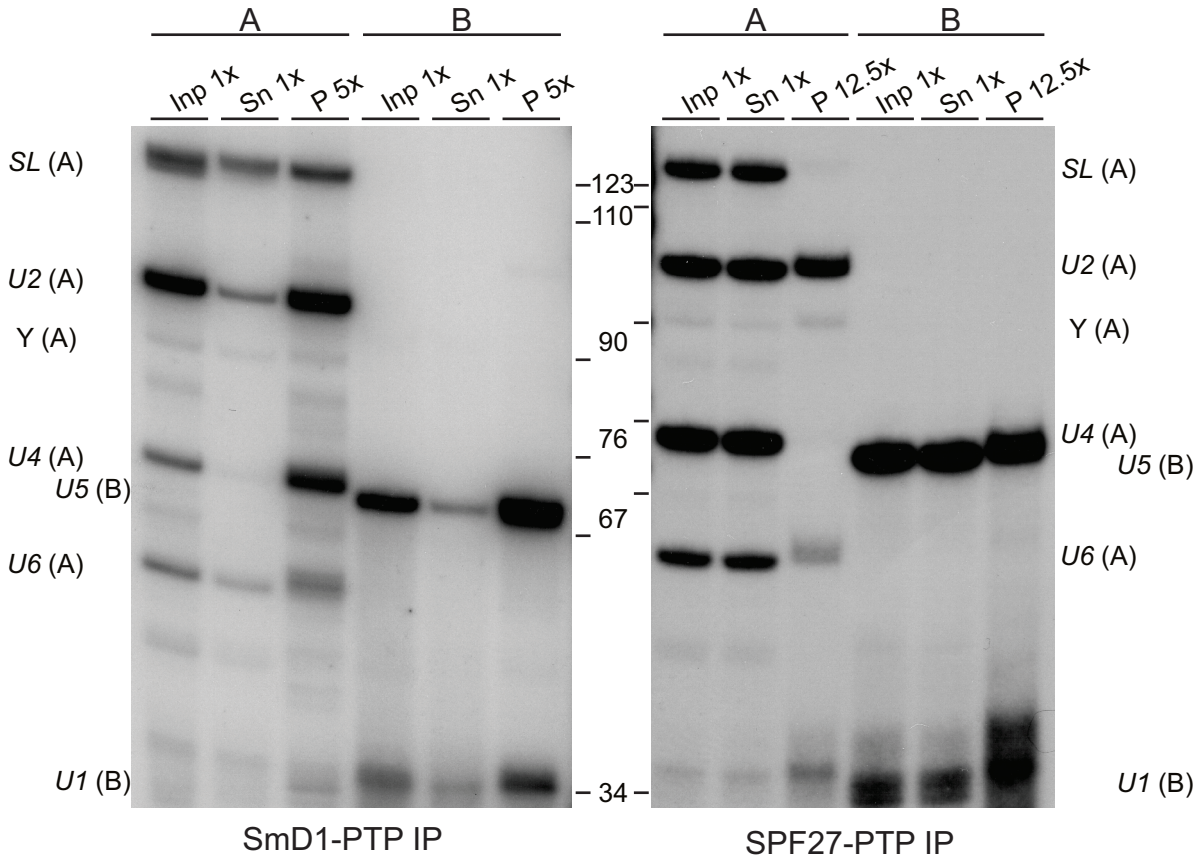

Fig. S4. Only a minor amount of spliceosomal U snRNAs are bound to the PRP19 complex in extract

Primer extension analysis of total RNA prepared from the SmD1-PTP and SPF27-PTP pull-downs documented in figure 6A. The analysis shows the snRNA profiles of crude extracts (Inp), supernatants (Sn) and precipitates (P) in reaction A which detects SL RNA, U2/U4/U6 snRNAs and the Y structure intermediate, and reaction B which detects U5/U1 snRNAs. x-Values indicate relative amounts loaded. Note that the high volume of the P sample for the SPF27 analysis (right panel) led to a slight upward shift of signals. Also note that the SmD1 and SPF27 reactions were separated on 6% and 8% polyacrylamide gels, respectively, leading to different spacing of primer extension products. Nevertheless, the results clearly show that SmD1 precipitation depleted U2, U4, U5, and U1 snRNAs and, to a lesser extent, SL RNA and U6 snRNA, whereas SPF27 precipitation did not lead to a detectable reduction of snRNAs. This indicates that, in extract, only a minor amount of snRNAs are stably associated with SPF27 in a spliceosomal complex.

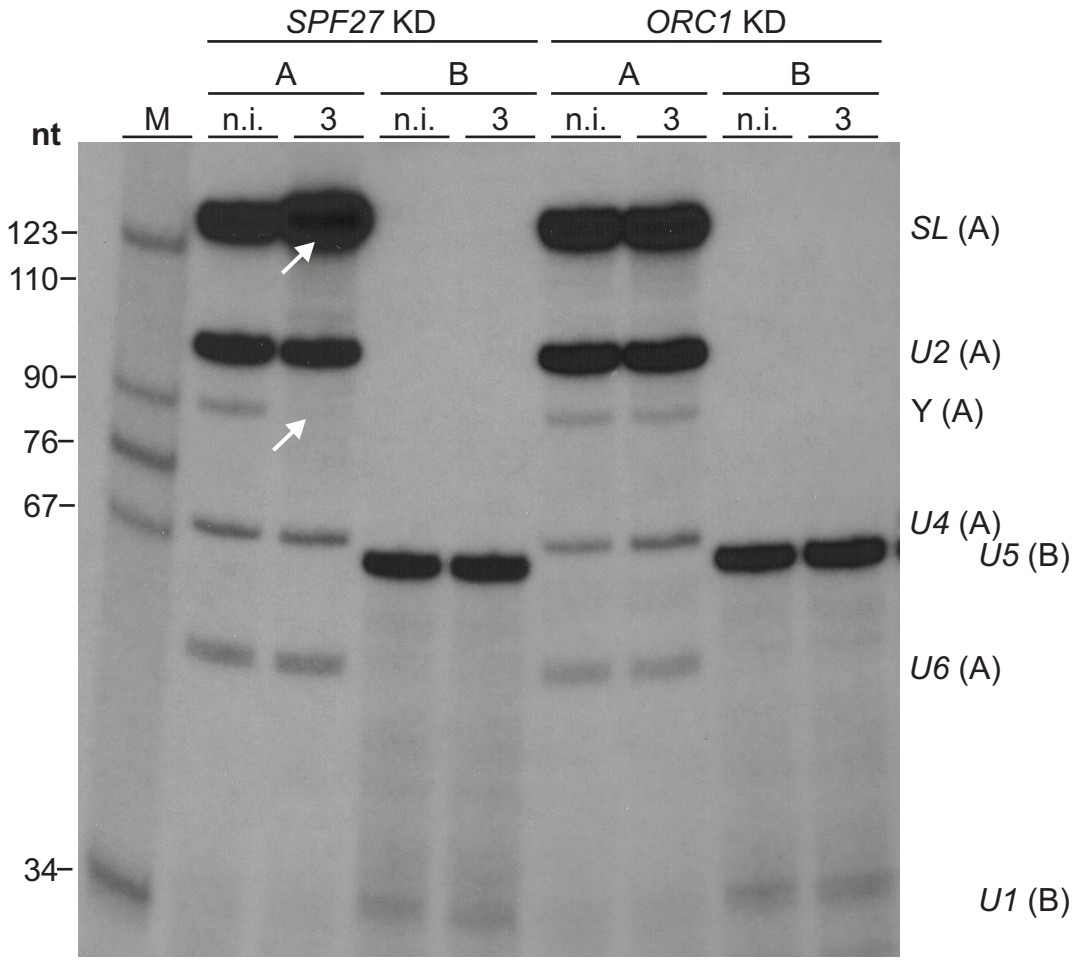

Fig. S5. Spliceosomal U snRNAs remain stable upon *TbSPF27* silencing

Total RNA was prepared from non-induced cells (n.i.) and from cells in which either *SPF27* or, as a control, *ORC1* was silenced (KD for knockdown) for 3 days. RNA abundances were determined by two primer extension assays. In assay A, ^{32}P-5′-endlabeled oligonucleotides were combined that specifically hybridize to SL RNA and U2, U4 and U6 snRNAs. In assay B, U1- and U5-specific oligonucleotides were used. Primer extension products were separated on 8% polyacrylamide-50% urea gels and visualized by autoradiography. M, marker *Msp*I-digested pBR322. On the right, SL RNA and U snRNA products are specified. Y denotes a product of the SL RNA-specific oligonucleotide that is generated by the Y structure intermediate after the first step of splicing. The arrows point to increased and decreased signals of SL RNA and Y structure intermediate in RNA of *SPF27*-silenced cells, respectively. Other signal strengths remained comparable confirming that the trypanosome PRP19 complex is a non-snRNP component of the spliceosome.

Supplemental Reference

McWilliam,H., Li,W., Uludag,M., Squizzato,S., Park,Y.M., Buso,N. *et al.* (2013) Analysis Tool Web Services from the EMBL-EBI. *Nucleic Acids Res* **41**: W597-W600.