Supplementary Material

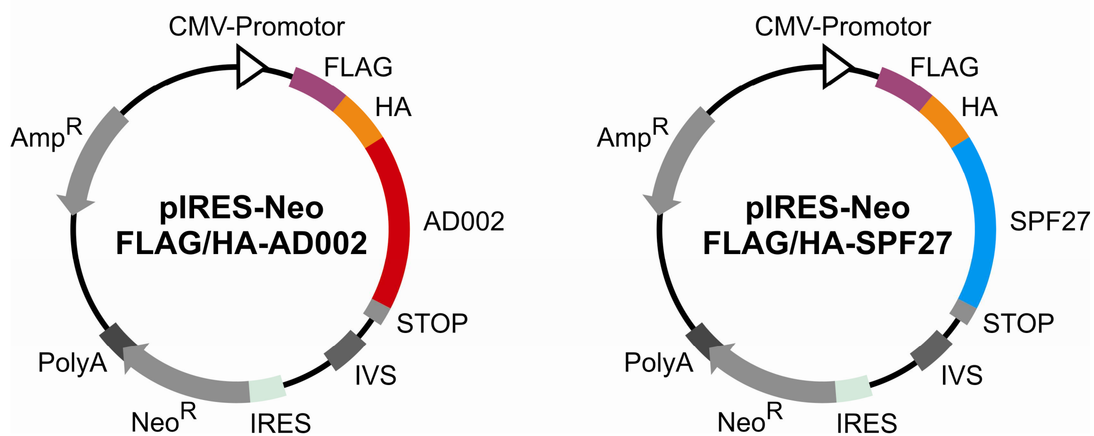
Oligonucleotides and cloning procedures

Oligo	Sequence
MG1	GGC CGC GGG CGG CAT CGA GGG CAG GGG CGG CGG CGG CTG GTC CAA CCC ACA ATT CGA GAA GCC CGG GTA ATA AC
MG2	CAT GGT TAT TAC CCG GGC TTC TCG AAT TGT GGG TGG GAC CAG CCG CCG CCG CCG CCC CTG CCC TCG ATG CCG CCC GC
MG3	CCG GAT ATC ATG ACA ACA GCA GCC AGG CC
MG4	AAG GAA AAA AGC GGC CGC TTT AAT ATA TTT CTC CAT GAA CTT TTT GTG
MG5	CCG GAT ATC ATG CCT CGA ATT ATG ATC AAG
MG6	AAG GAA AAA AGC GGC CGC GAA TTT TGA CTT TAA AGT CTC TTT CTC
MG7	CCG GAT ATC ATG GAC GTG GGC GAA CTT CTG
MG8	CTT CCC GAT AGC GTC CGC GTT CTC GAG
MG9	CTC GAG AAC GCG GAC GCT ATC GGG AAG
MG10	ATA AGA ATG CGG CCG CGA AGT TCT CCA GCA AGC CCA G
MG11	CCG GAT ATC ATG TCC CTA ATC TGC TCC ATC TCT AAC
MG12	AAG GAA AAA AGC GGC CGC CAG GCT GTA GAA CTT GAG GCT TCT GTC
MG13	CCG GAT ATC ATG GTC GAG GAG GTA CAG AAA C
MG14	AAG GAA AAA AGC GGC CGC AAA TCT CTT TCT CTT GAT AAT TTC TG
MG15	CCG GAT ATC TCC AAG GGA CCT GCA GTT G
MG16	AAG GAA AAA AGC GGC CGC ATC AAC CTC TTC AAT GGT GGG C
MG17	CCG GAT ATC ATG GCG GGC ACA GGT TTG GTG
MG18	AAG GAA AAA AGC GGC CGC GAA GTC TTG CCG GAT GTT TTC TTT G
IL1	TAC GCT AGC ATG ACA ACA GCA GCC AGG CC
IL2	CTA GTG GAT CCC TAT TTA ATA TAT TTC TCC ATG
IL3	TAC GCT AGC ATG GCG GGC ACA GGT TTG G
IL4	CTA GTG GAT CCC TAG AAG TCT TGC CGG ATG TTT TC
M7-F	GGG GTA CCT AAT ACG ACT CAC TAT AGG GAG ACG GAA TTC GAG CTC GCC C
M7-R	CGC GGA TCC CCA CTG GAA AGA CC

To generate FLAG/HA-tagged AD002 and SPF27, cDNAs encoding these proteins were cloned into a modified pIRESneo plasmid (Clontech) containing a N-terminal FLAG/HA tag (2) using PCR based techniques. The AD002 and SPF27 cDNAs were cloned using Nhe I and BamH I restriction sites, with IL1/IL2 and IL3/IL4 primer pairs. In order to introduce a StrepII purification tag (4), oligonucleotides MG1 and

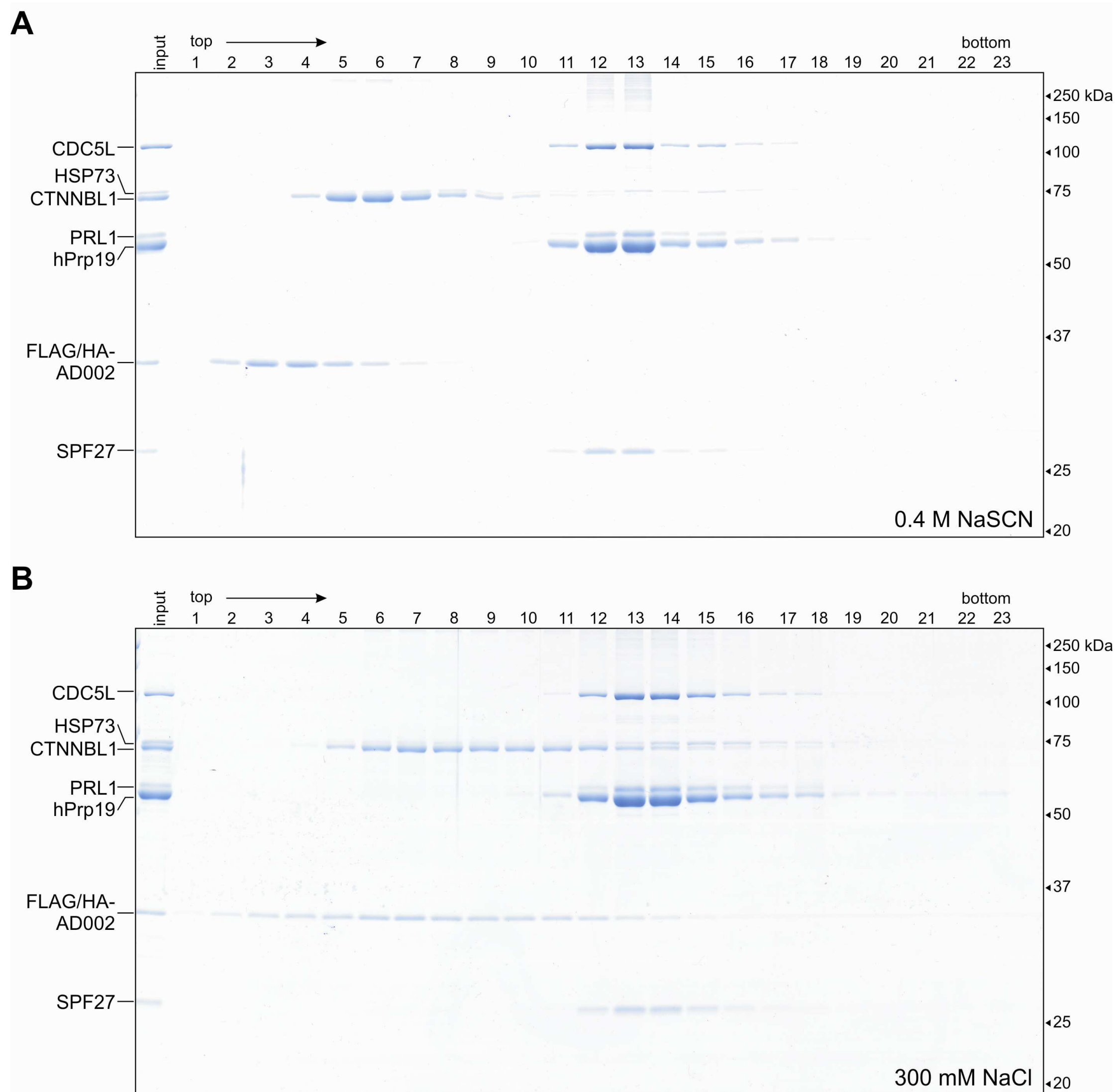
MG2 were hybridized and cloned via Not I and Nco I cleavage sites into the pEU3-NII vector (Toyobo) (3), generating pEU3-NII-StrepII. The cDNAs encoding all seven hPrp19/CDC5L complex proteins were cloned into pEU3-NII-StrepII via EcoRV and Not I cleavage sites using primers MG3-MG18.

Supplementary Figure 1



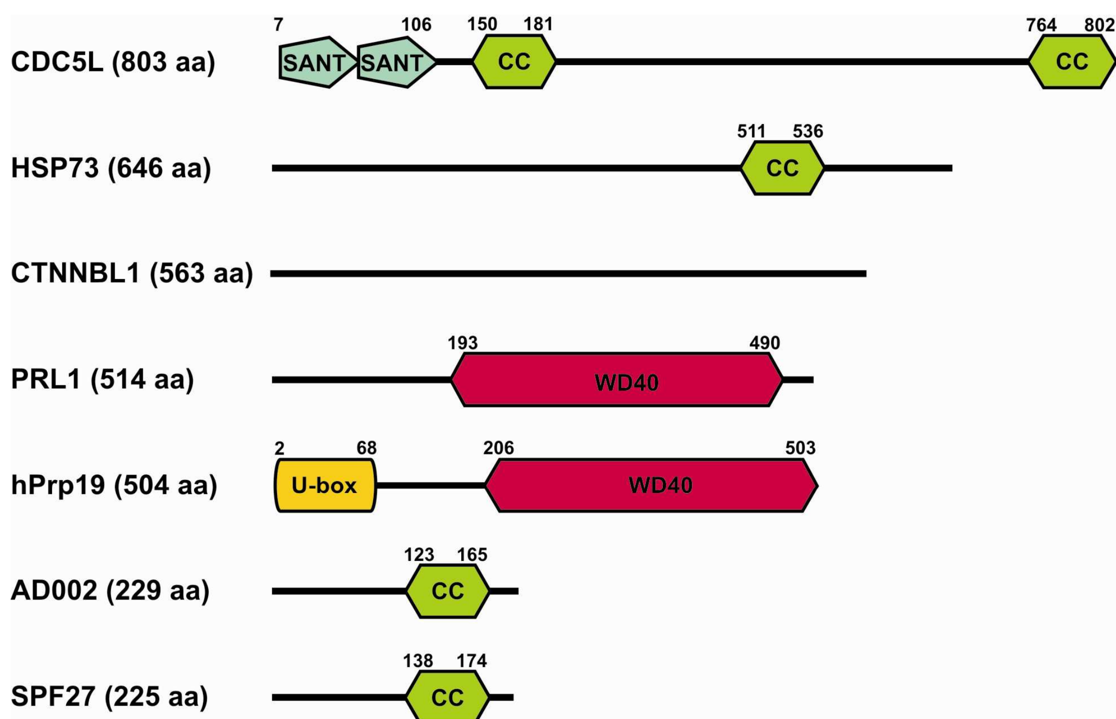
Schematic illustration of plasmids used for the stable transfection of HeLa cells. The plasmids contain the FLAG/HA-tagged AD002 or SPF27 genes under the control of a CMV promotor. In addition, the plasmids contain ampicillin and neomycine resistance markers for selection in bacteria and mammalian cells, respectively. IVS – intervening sequence; IRES – internal ribosome entry site.

Supplementary Figure 2



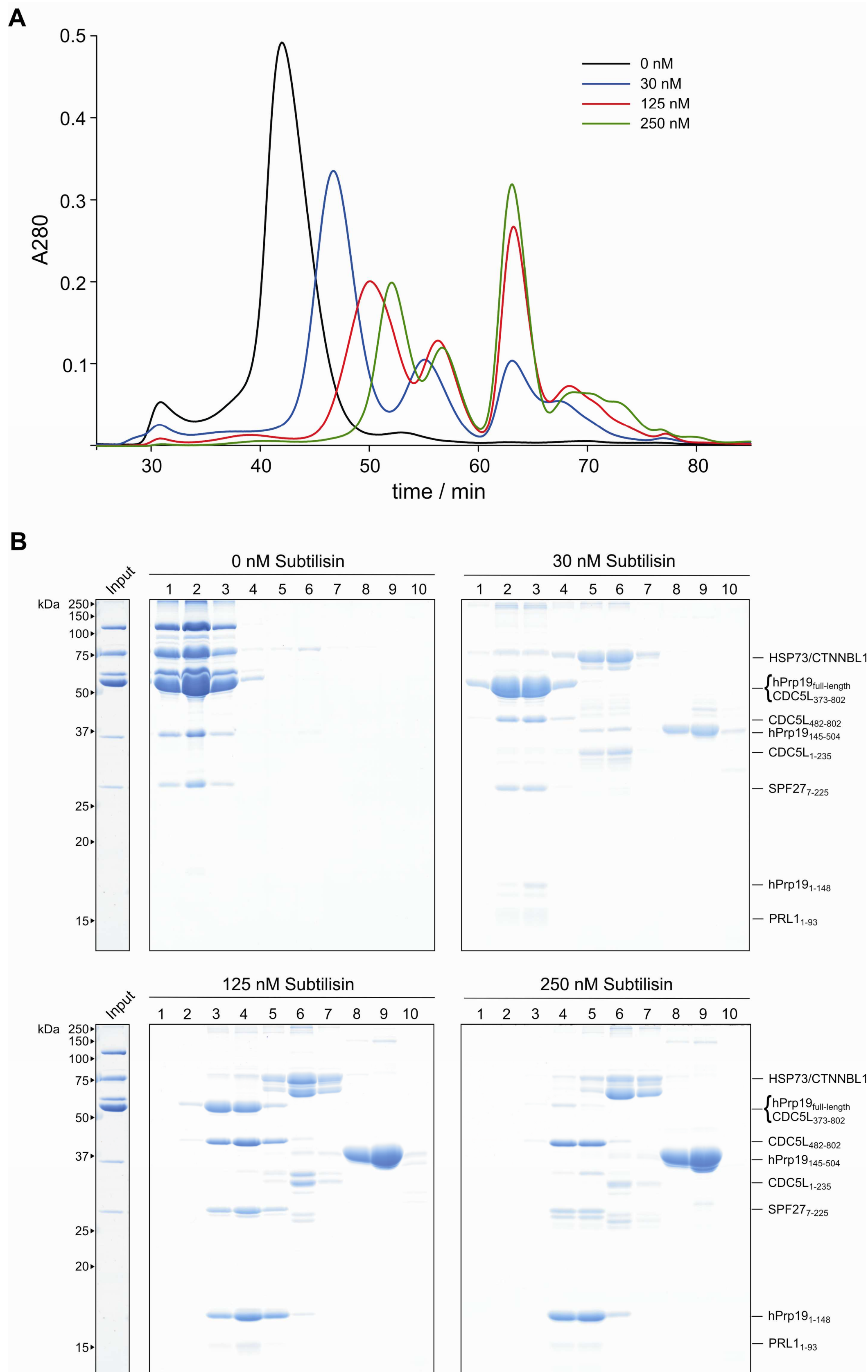
CDC5L, PRL1, hPrp19, and SPF27 form a salt-stable subcomplex. Purified hPrp19/CDC5L complexes were incubated with 0.4 M NaSCN (A) or 300 mM NaCl (B) for 30 min on ice. Subsequently, the complexes were fractionated on a linear 5 to 20% glycerol gradient containing the same NaSCN or NaCl concentration. Proteins across the gradient were analyzed by SDS-PAGE and stained with Coomassie. The position of the proteins is indicated on the left and the molecular mass (kDa) of marker proteins is indicated on the right.

Supplementary Figure 3



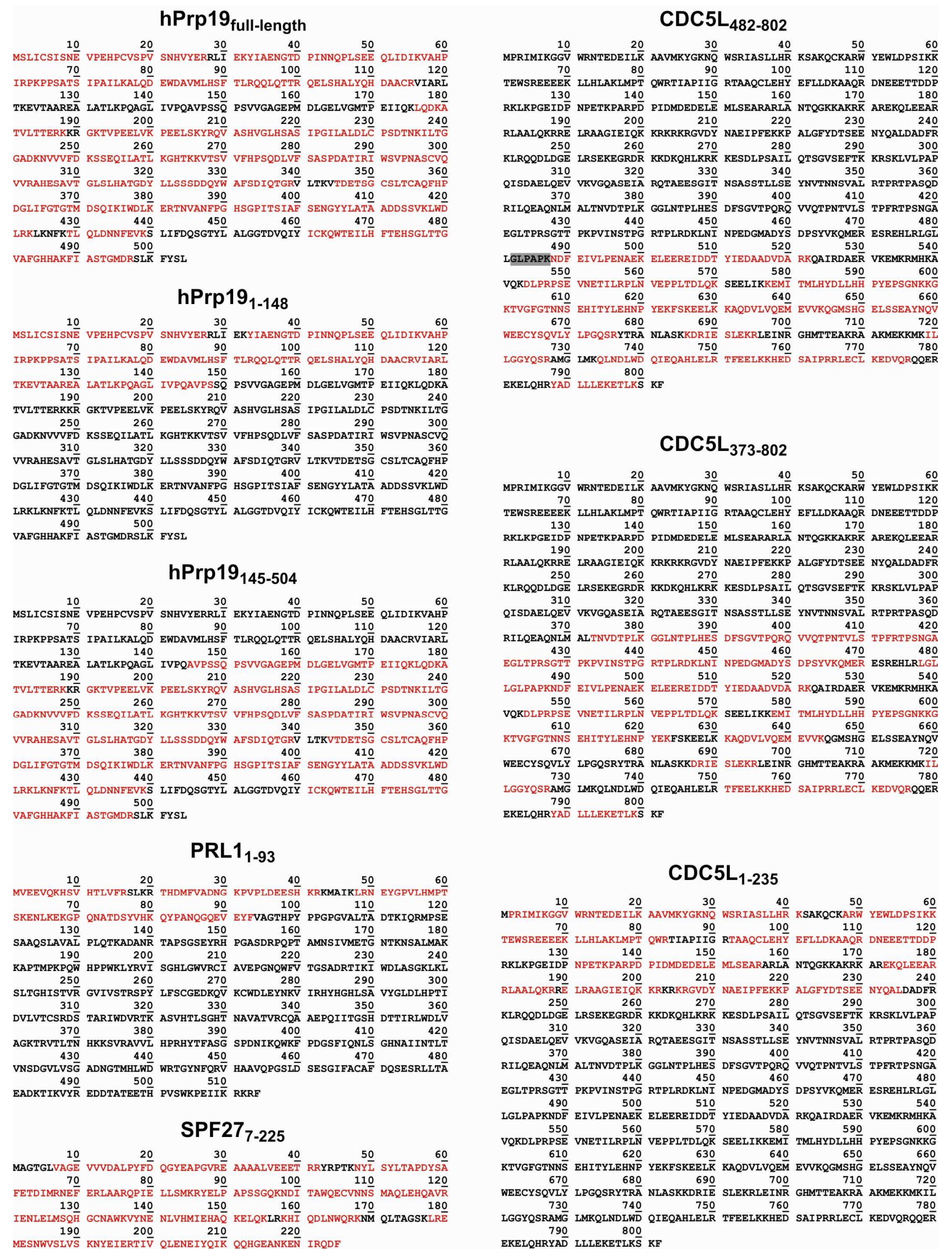
Schematic overview of domains in the hPrp19/CDC5L complex proteins. The total number of amino acids (in parenthesis) and the position of domains predicted by the SMART 6 program (1) are indicated. The WD40 domains of hPrp19 and PRL1 both consist of 7 WD40 repeats. CC – Coiled Coil domain; SANT (also cMyb) – Switching-defective protein 3 (Swi3), Adaptor 2 (Ada2), Nuclear receptor co-repressor (N-CoR), Transcription factor (TF)IIIB.

Supplementary Figure 4



Limited proteolysis with subtilisin reveals the architecture of the hPrp19/CDC5L complex core. Affinity-selected complexes were incubated for 30 min on ice with 0, 30, 125, or 250 nM of the protease subtilisin. The proteolysed fragments were then fractionated by size via gel filtration and analysed by SDS-PAGE followed by mass spectrometry in order to define those fragments resistant to protease digestion. (**A**) Gel filtration profile after proteolysis with 0 to 250 nM subtilisin (as indicated). (B) SDS-PAGE analysis of subtilisin digestion products co-purifying during gel filtration. Proteins from pairwise pooled fractions were separated on a 15% polyacrylamide-SDS gel, stained with coomassie and analyzed by mass spectrometry. The protein residues remaining after proteolysis, as determined by mass spectrometry, are indicated in parentheses.

Supplementary Figure 5



Exhaustive mass spectrometric analyses of hPrp19/CDC5L complex proteins after limited proteolysis and subsequent trypsin digestion. The peptides identified via MALDI-MS/MS are highlighted red. The presence of the N-terminal peptide of CDC5L (482-802), indicated with a grey box, was verified by N-terminal Edman sequencing.

Supplementary references

1. **Letunic, I., T. Doerks, and P. Bork.** 2009. SMART 6: recent updates and new developments. Nucleic Acids Res. **37:** 229-232.
2. **Malik, S., and R. G. Roeder.** 2003. Isolation and functional characterization of the TRAP/mediator complex. Methods Enzymol. **364:**257-284.
3. **Sawasaki, T., T. Ogasawara, R. Morishita, and Y. Endo.** 2002. A cell-free protein synthesis system for high-throughput proteomics. Proc. Natl. Acad. Sci. U S A **99:**14652-14657.
4. **Voss, S., and A. Skerra.** 1997. Mutagenesis of a flexible loop in streptavidin leads to higher affinity for the Strep-tag II peptide and improved performance in recombinant protein purification. Protein Eng. **10:**975-982.