

Figure S1

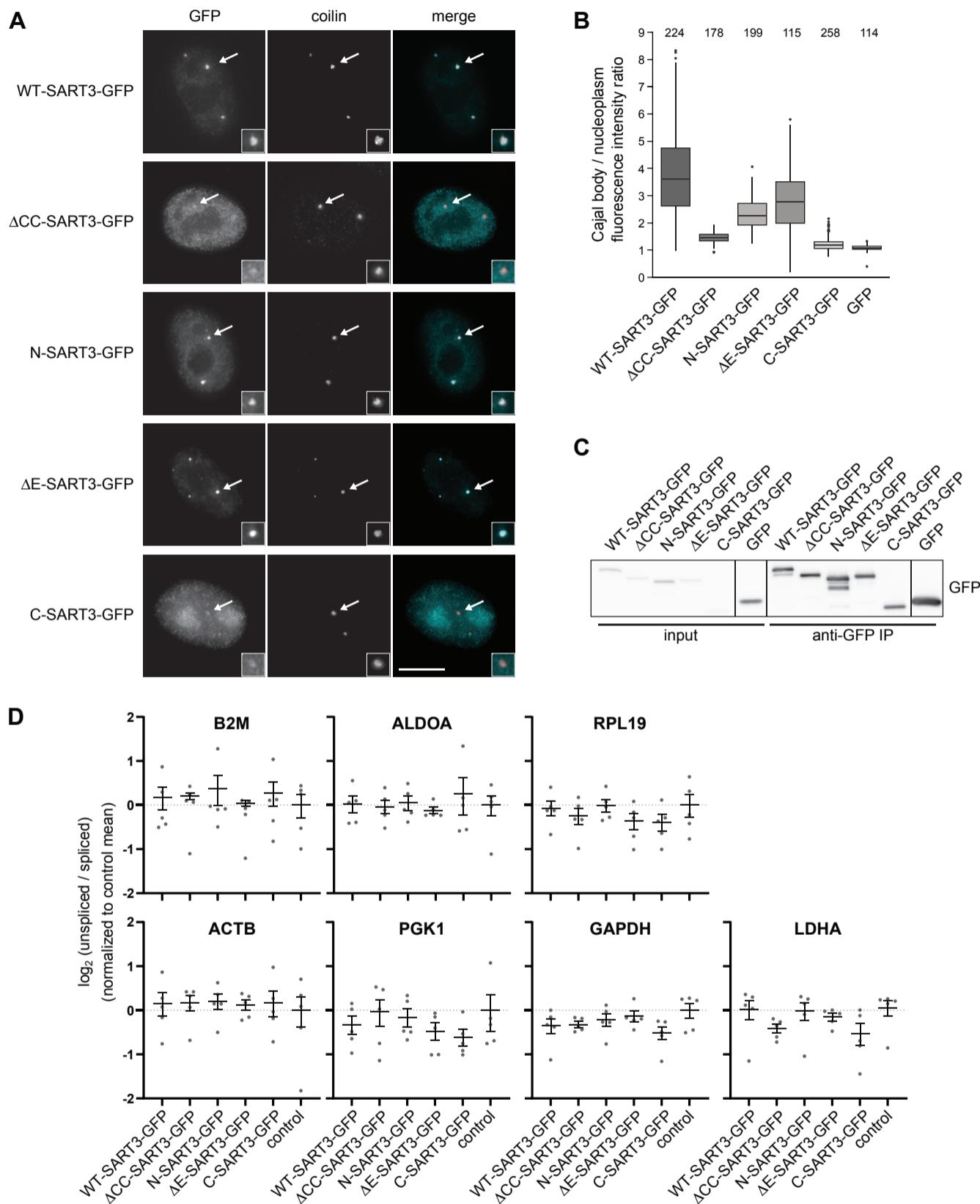


Fig. S1. Localization and expression of SART3 variants. (A) SART3-GFP constructs (see Fig. 1A) were transiently expressed in HeLa cells and their localization monitored by fluorescence microscopy (A). Coilin, a marker of Cajal bodies was visualized by indirect immunofluorescence. Cajal bodies marked by arrows were two-fold magnified and are shown in insets. In merged images, coilin is shown in red, GFP fluorescence in turquoise and the scale bar represents 10 µm. (B) Accumulation of SART3-GFP constructs in Cajal bodies was quantified by measuring GFP intensities in the Cajal body and the nucleoplasm. Number of Cajal bodies assayed is indicated above the graph. Statistical significance was tested by two-tailed, unpaired t-test. Changes are significant in all cases with $p < 0.001$. (C) Expression level of SART3-GFP constructs in cell lysates and after immunoprecipitation with anti-GFP antibodies was monitored by western blotting using the mouse anti-GFP antibody. (D) SART3-GFP constructs were transiently expressed in HeLa cells and unsplited/spliced products of selected genes were monitored by reverse transcription followed by quantitative PCR. Mean and s.e.m. from five biological replicates are shown.

Figure S2

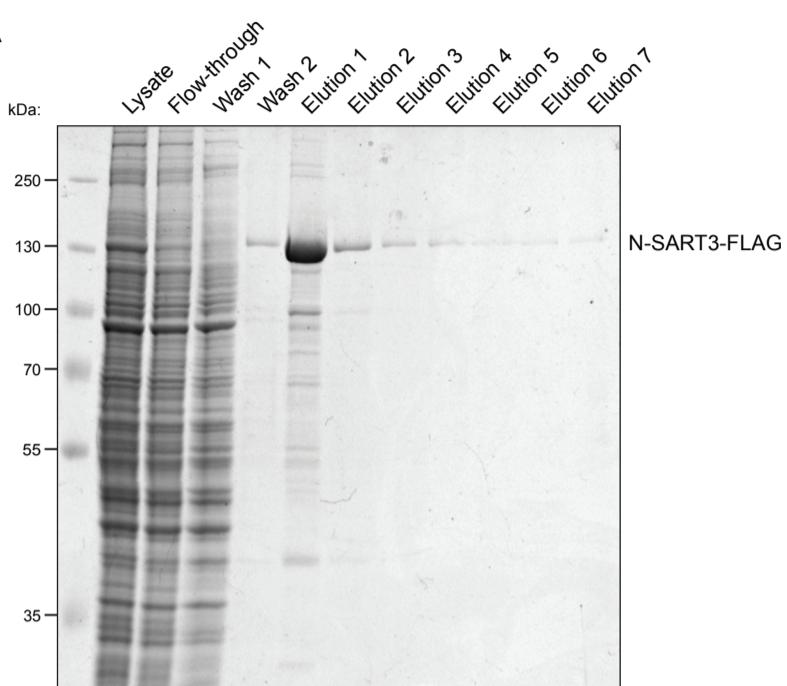
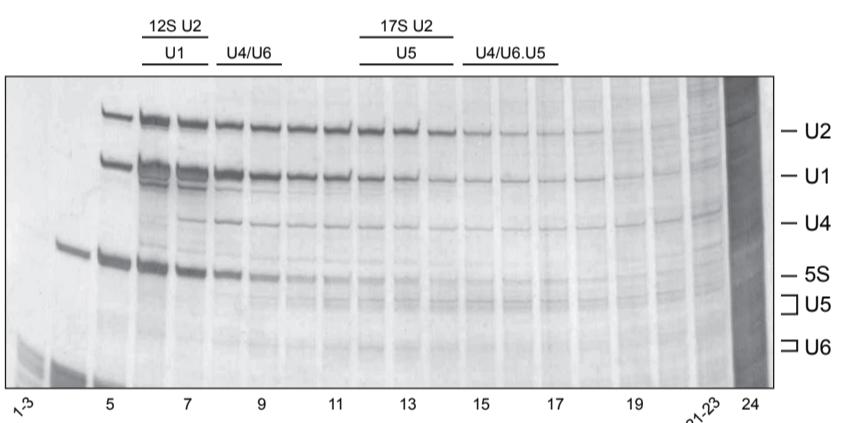
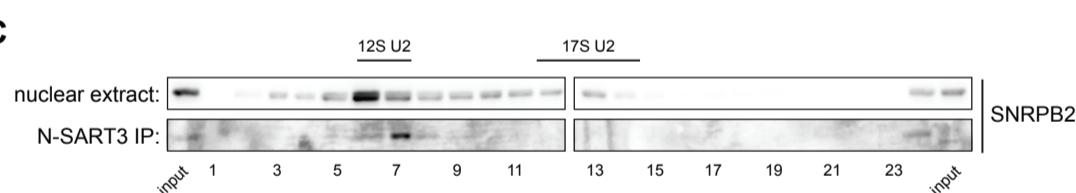
A**B****C**

Fig. S2. Isolation of recombinant N-SART3 and monitoring distribution of snRNAs in glycerol gradient after ultracentrifugation. (A) A Coomassie blue stained polyacrylamide gel showing isolation of recombinant N-SART3- Strep-FLAG-HALO. (B) Nuclear extract was subjected to ultracentrifugation in 10-30% glycerol gradient, RNA from individual fractions isolated, resolved on denaturing UREA polyacrylamide gels and silver stained. Position of individual snRNP particles is indicated above the gel. (C) N-SART3-FLAG was transiently expressed in HeLa cells and immunoprecipitated with the anti-FLAG antibody. Co-precipitated complexes were eluted by the FLAG peptide and resolved by ultracentrifugation in 10-30% glycerol gradient. Proteins were isolated from individual fractions and SNRPB2 detected by western blotting.

Figure S3

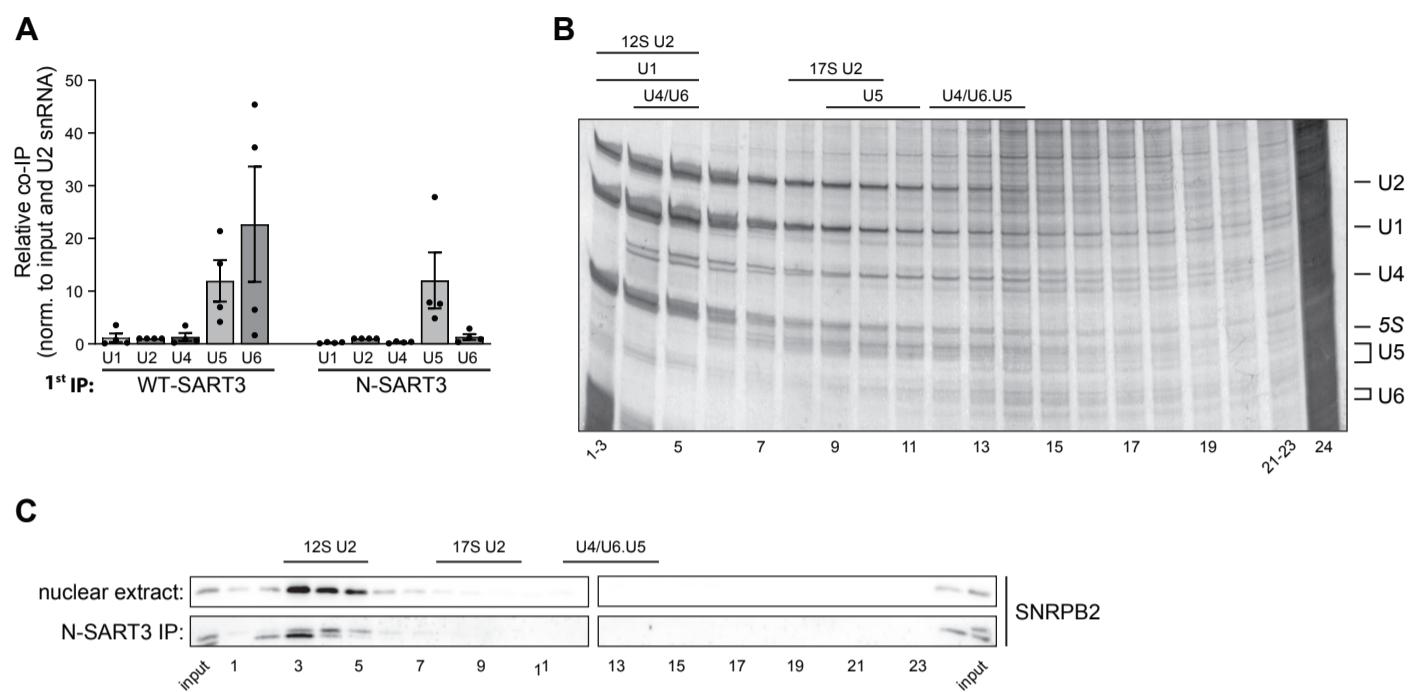


Fig. S3. snRNA detection in SART3-U2 complexes and monitoring distribution of snRNAs in glycerol gradient after ultracentrifugation. (A) snRNA co-purified by double immunoprecipitation with anti-GFP and anti-SNRPA1 antibodies were analyzed by RT-qPCR and double normalized to inputs and the U2 snRNA signal. (B) Nuclear extract was subjected to ultracentrifugation in 10–30% glycerol gradient, RNA from individual fractions isolated, resolved on denaturing UREA polyacrylamide gels and silver stained. Position of individual snRNP particles is indicated above the gel. (C) N-SART3-FLAG was transiently expressed in HeLa cells and immunoprecipitated with the anti-FLAG antibody. Co-precipitated complexes were eluted by the FLAG peptide and resolved by ultracentrifugation in 10–30% glycerol gradient. Proteins were isolated from individual fractions and SNRPB2 detected by western blotting.

Fig. S4. Western Blot Transparency

Fig.1D

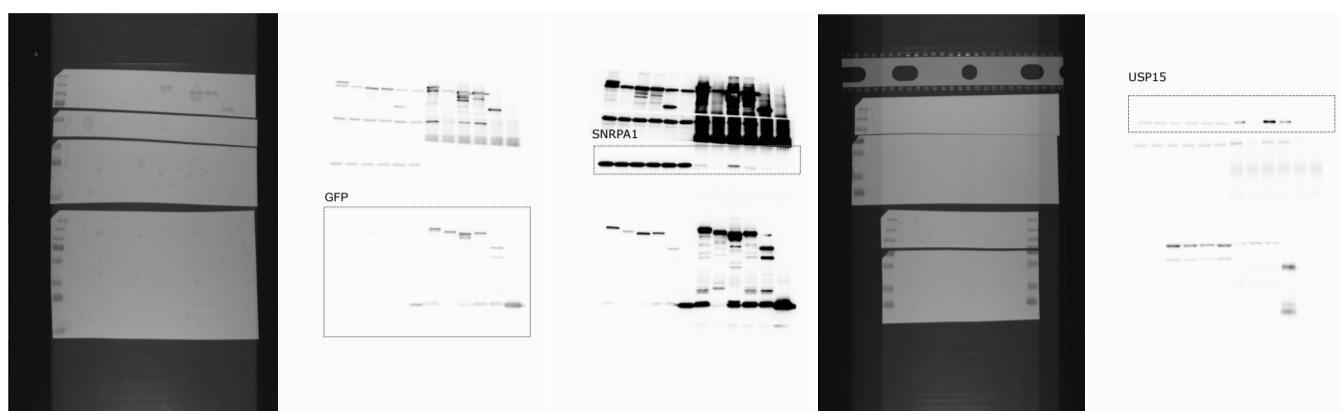


Fig.1E

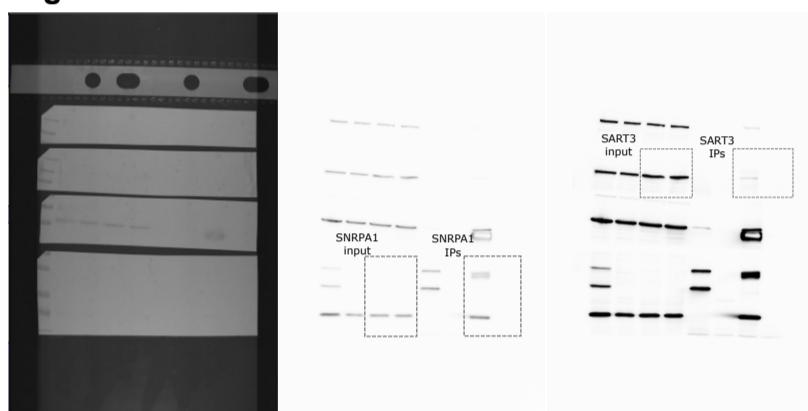


Fig.2A

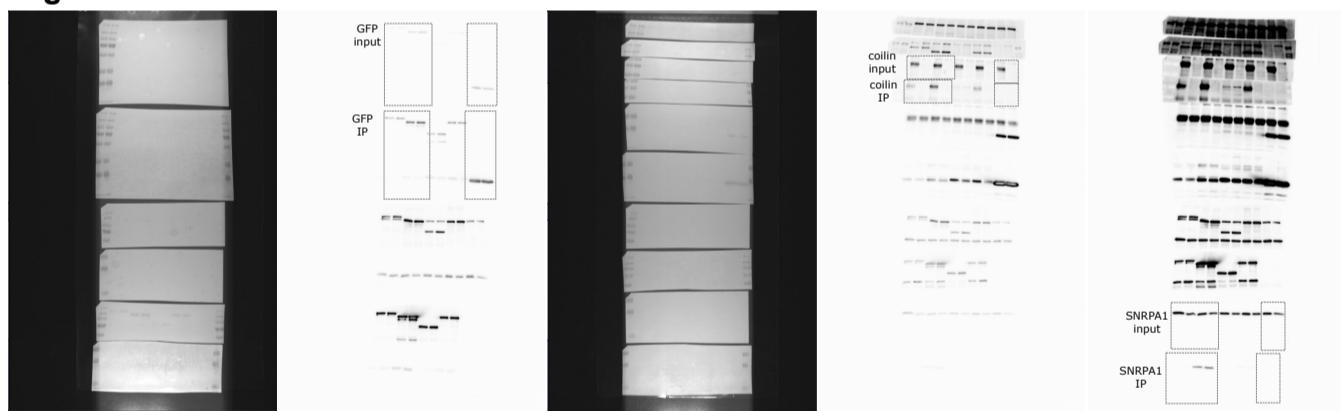


Fig.2B



Fig.2B - continued

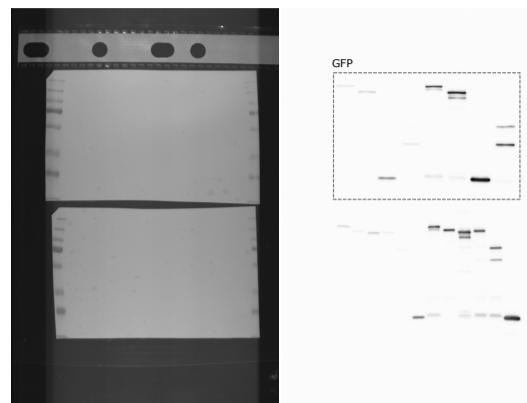


Fig.2C

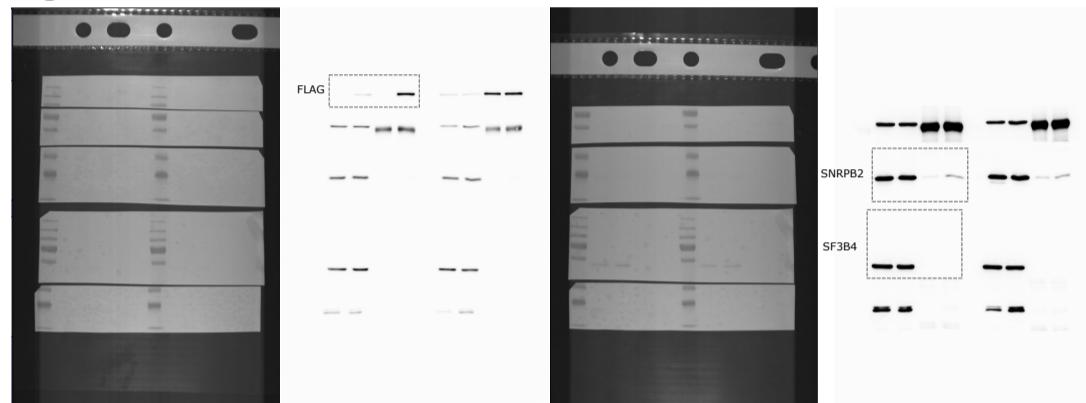


Fig.2D

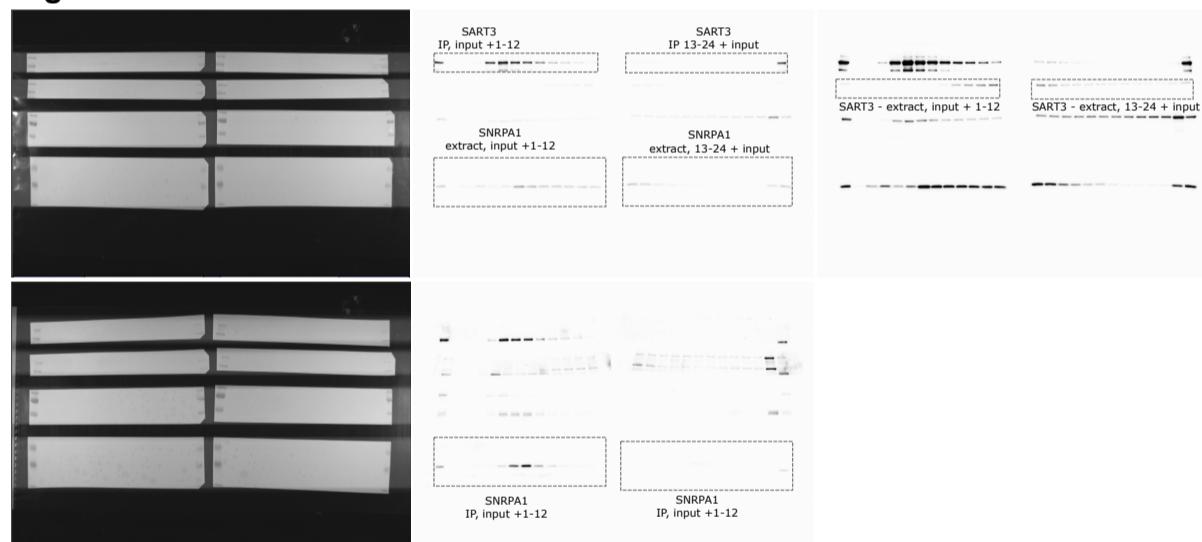


Fig.2E

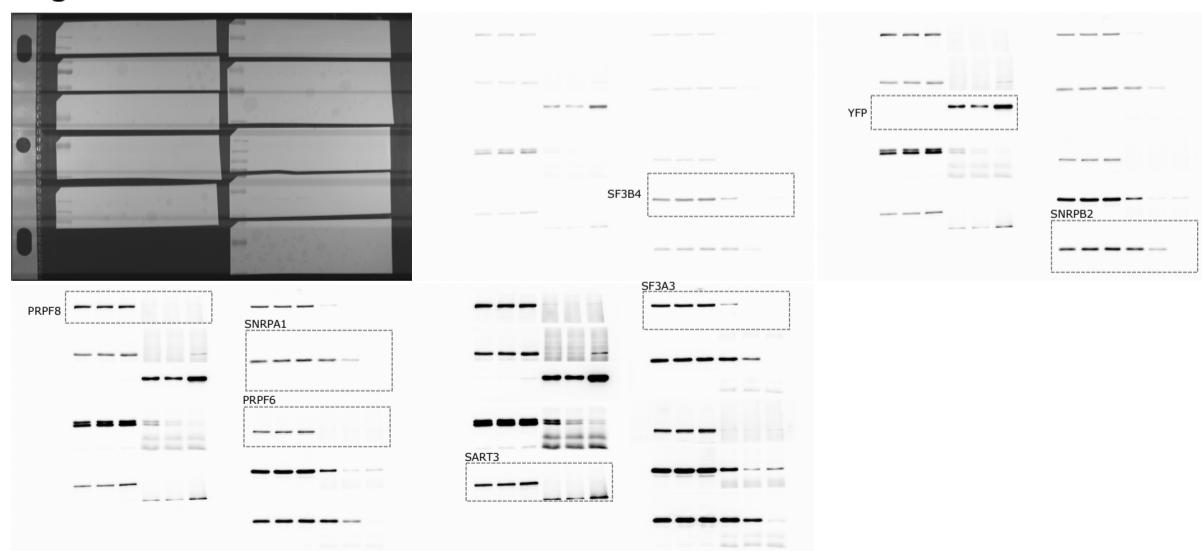


Fig.3A

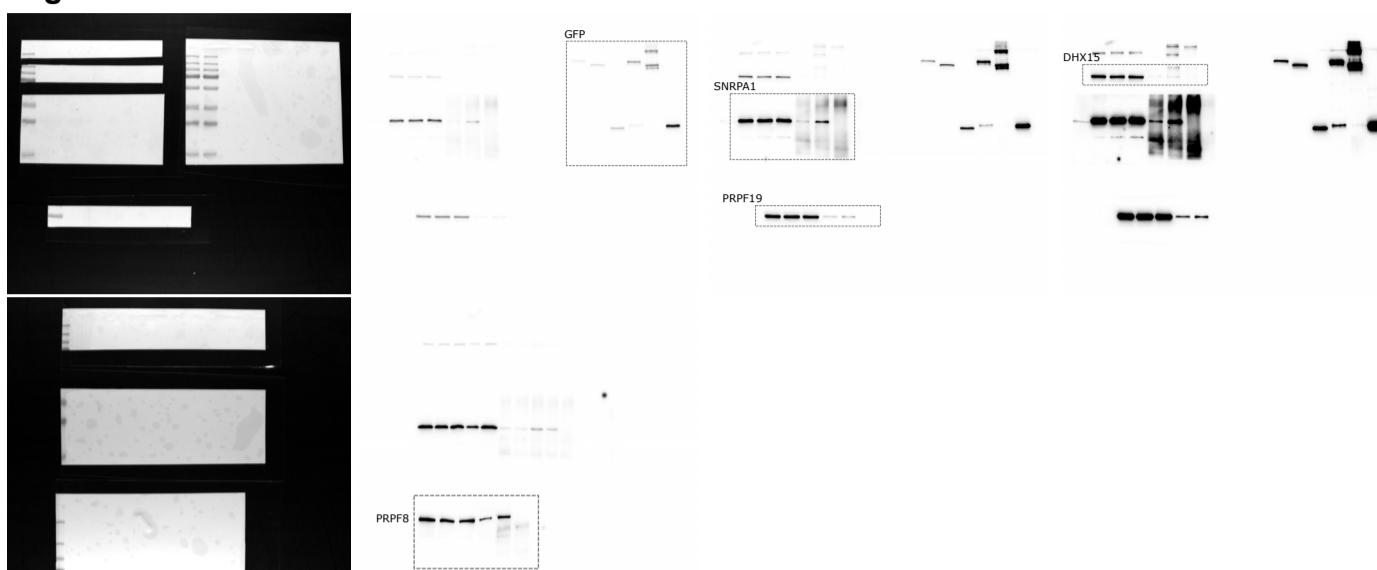


Fig.3B



Fig.3D

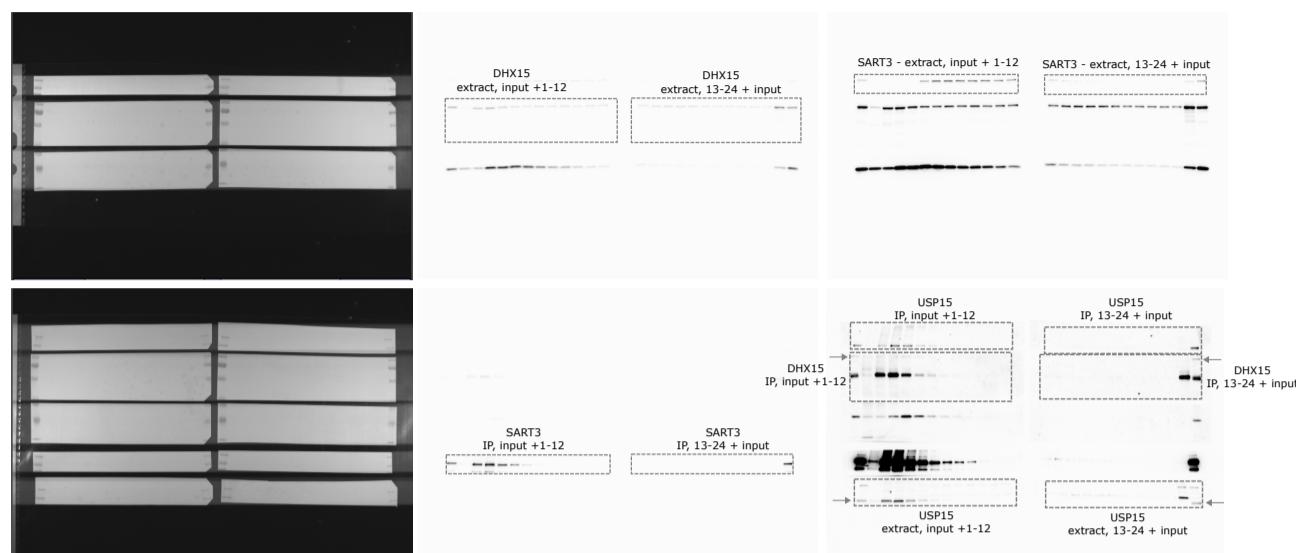


Fig.4A

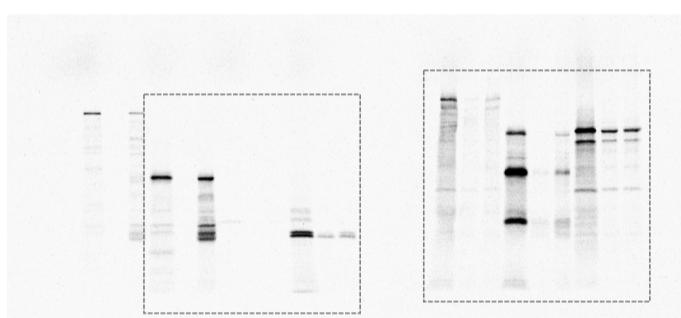


Fig.4B



Fig.4C

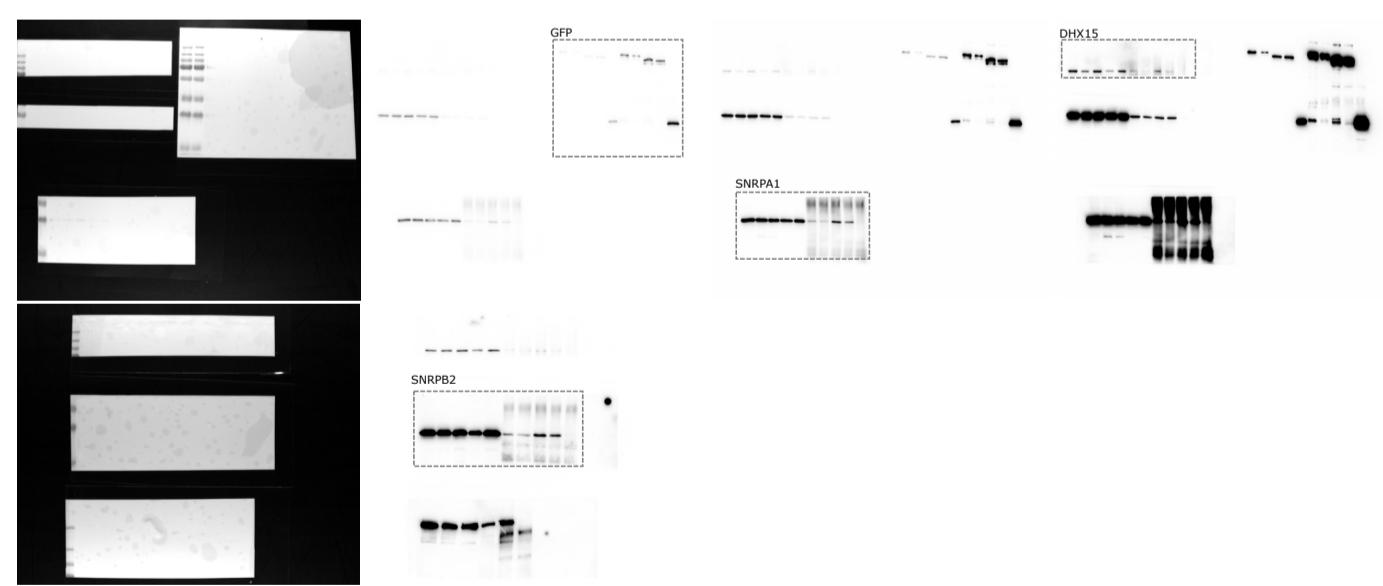


Fig.4D

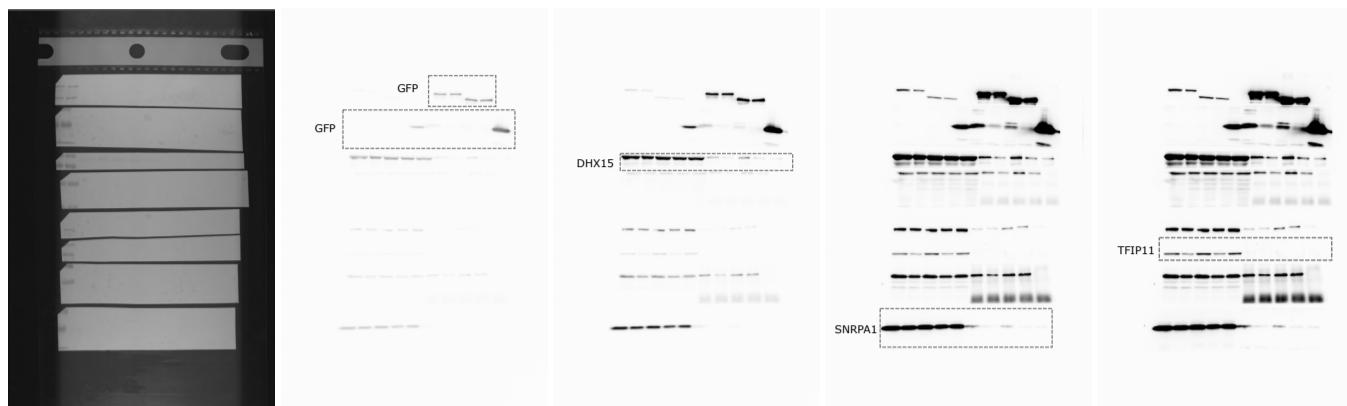


Fig.4E

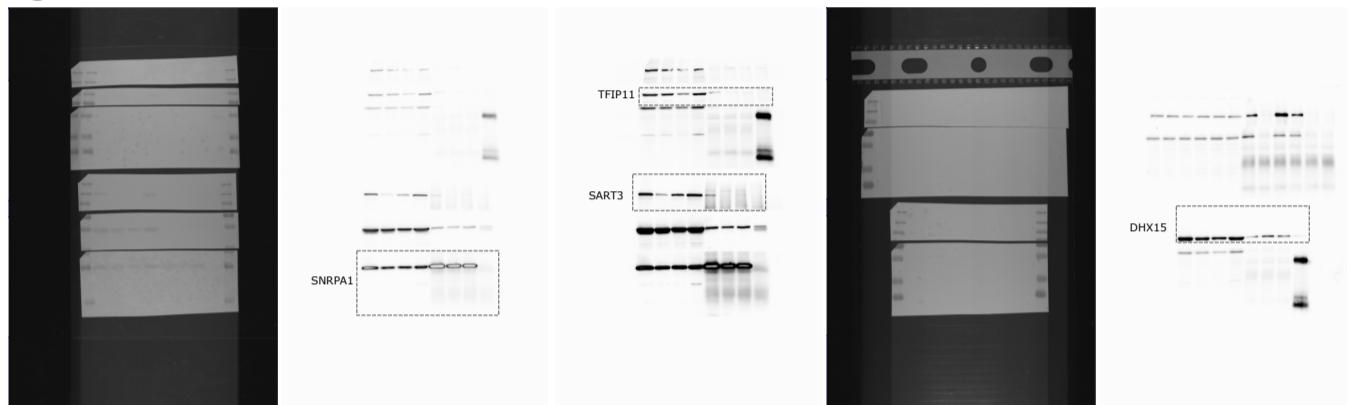


Fig.S1C

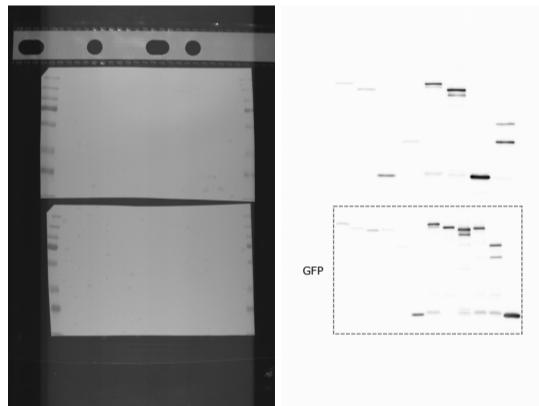


Fig.S2C

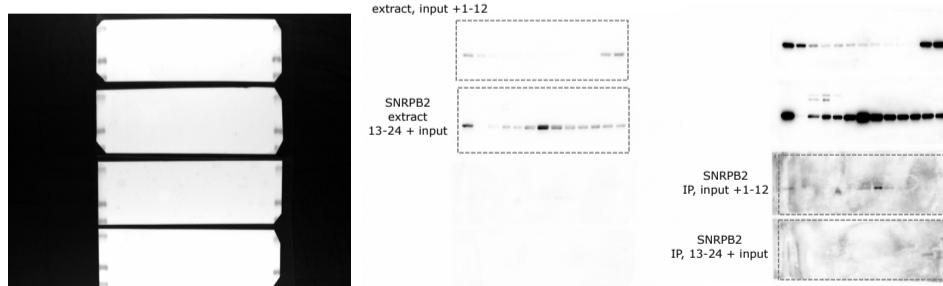


Fig.S3C

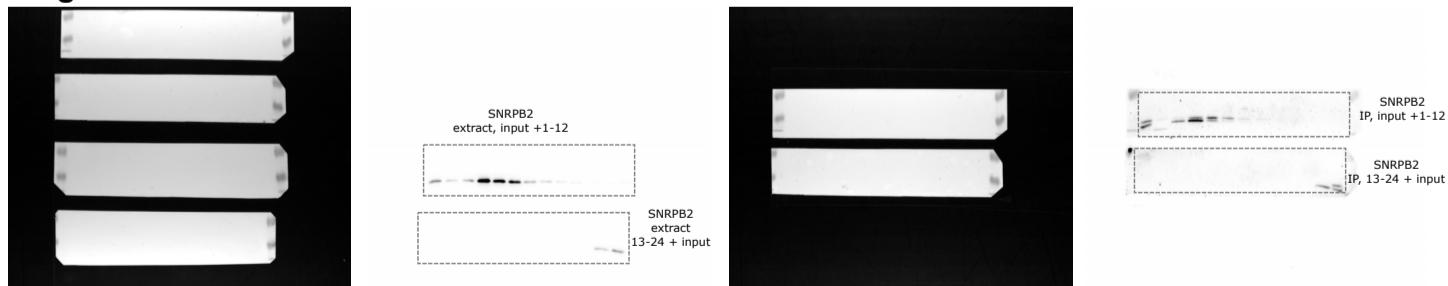


Table S1. A list of primers

Cloning primers

ΔE-SART3-pEGFP-N1	F: 5'-AACAGCTGGAGATTGAGAGACTG-3' R: 5'-CATGAGATCTGAGTCCGGTAGC-3'
SART3-TEV-pEGFP-N1	F: 5'-TTCCAGGGCGCCGGATCCACCGGTGCG-3' R: 5'-GTACAGGTTCTCCCGGGTACCGTCGACTGCA-3'
N-SART3-pFLAG-C3	F: 5'-GACGATGACAAGCAGTACTCAGATCTATGGCGACTGCGGCCGAAACCT-3' R: 5'-GATCCGGGCCGCGGTACCGTCGACTGCAGAAT-3'
N-SART3-pDONR221	F: 5'-GGGGACAAGTTGTACAAAAAAGCAGGCTCGATGGCGACTGCGGCCGAA-3' R: 5'-GGGGACCACTTGATGGAGATCAGACCAAATCACAA-3'
SNRPB2-pIVT	F: 5'-GACCTCGAGATGGATATCAGACCAAATCACAA-3' R: 5'-GACGAATTCTTATTCTGGATAGGTGATCTTC-3'
SNRPA1-pIVT	F: 5'-GACCTCGAGATGGTCAAGCTGACGGCG-3' R: 5'-GACGAATTCTCAGGACCCGTTGTGACTGTG-3'

PCR primers for TnT templates

PRPF3-383-683-TnT	F: 5'-TACGTAATACGACTCACTATAGGGAGAGCCACCATTGGATATTCTGAAATTGAGTGG-3' R: 5'-GACGTCGACTCAATCAGTGGACTCTAACACAG-3'
DHX15-TnT	F: 5'-TACGTAATACGACTCACTATAGGGAGAGCCACCATGTCCAAGCGGCACCGGTTG-3' R: 5'-GACGAATTCTCAGTACTGTGAATATTCTGGATTG-3'
DHX15-1-320-TnT	F: 5'-TACGTAATACGACTCACTATAGGGAGAGCCACCATGTCCAAGCGGCACCGGTTG-3' R: 5'-TCACTCAACAGGATGTGACGCC-3'
DHX15-288-795-TnT	F: 5'-TACGTAATACGACTCACTATAGGGAGAGccACATGGTTAGTTATGAGCGCTACTC-3' R: 5'-GACGAATTCTCAGTACTGTGAATATTCTGGATTG-3'

qPCR primers

U1 snRNA	F: 5'-ATACTTACCTGGCAGGGGAG-3' R: 5'-CAGGGAAAGCGCGAACGCA-3'
U2 snRNA	F: 5'-CTGGCCTTTGGCTAAGAT-3' R: 5'-CGTCCCTGGAGGTACTGCAA-3'
U4 snRNA	F: 5'-TGGCAGTATCGTAGCAAATG-3' R: 5'-CTGTCAAAAATTGCCAGTGC-3'
U5 snRNA	F: 5'-CTCTGGTTCTCTCAGATC-3' R: 5'-TGTTCCCTCCACGGAAATC-3'
U6 snRNA	F: 5'-CGCTTCGGCAGCACATATAC-3' R: 5'-AAAATATGGAACGCTCACGA-3'
ACTB spliced	F: 5'-CGTGCCTGACATTAAGGAGA-3' R: 5'-ACAGGACTCCATGCCAG-3'
ACTB unspliced	F: 5'-AGCTAAGTCTGCCCTCATT-3' R: 5'-GTACAGGTCTTGGATGT-3'
ALDOA spliced	F: 5'-TATCAAATCCAAGGGCGGTG-3' R: 5'-GCTCCGTCTTGTACTG-3'
ALDOA unspliced	F: 5'-TATCAAATCCAAGGGCGGTG-3' R: 5'-ATTCCCTGCCTCACTAACCT-3'
B2M spliced	F: 5'-AGATGTCTCGCTCCGTGG-3'

	R: 5'-CGTGAGTAAACCTGAATCTTGG-3'
B2M unspliced	F: 5'-AGATGTCTCGCTCCGTGG-3' R: 5'-CTTGGAGAAGGGAAGTCACG-3'
GAPDH spliced	F: 5'-ACATCGCTCAGACACCATGG-3' R: 5'-GTTAAAAGCAGCCCTGGTGA-3'
GAPDH unspliced	F: 5'-CAGGGAAGCTCAAGGGAGAT-3' R: 5'-GTTAAAAGCAGCCCTGGTGA-3'
LDHA spliced	F: 5'-TGGCAGCCTTCCCTTAGAA-3' R: 5'-CTTCTCCCTCTGCTGACG-3'
LDHA unspliced	F: 5'-TGGCAGCCTTCCCTTAGAA-3' R: 5'-TGTGCAACTGCACTTACCC-3'
PGK1 spliced	F: 5'-ACAACCAGATAACAAACACCAG-3' R: 5'-GAGTACTTGTCAAGGCATGGG-3'
PGK1 unspliced	F: 5'-TGTTGTCTCTTTGGTTGCA-3' R: 5'-GAGTACTTGTCAAGGCATGGG-3'
RPL19 spliced	F: 5'-ATGCCAGAGAAGGTACATG-3' R: 5'-CACATTCCCCTCACCTTC-3'
RPL19 unspliced	F: 5'-ATGCCAGAGAAGGTACATG-3' R: 5'-ACTAGCCATCAAAGCAGCAA-3'