

Figure S1: mCherry-SNRPN exhibits similar behaviour to both YFP-SNRPN and SNRPB in SH-SY5Y cells. A) SH-SY5Y cells transiently expressing mCherry-SNRPN and fixed after 24, 48 and 72 hours show variations in distribution of the mCherry-SNRPN with time. Immunostaining with Y12 (green on overlay) and anti-coilin (white on overlay) shows splicing speckles (arrowheads) and Cajal Bodies (CBs, triangles) respectively. Images are deconvolved z-stacks with 0.2 μ m spacing. Bar=7 μ m. B) mCherry-SNRPN initially localises diffusely in the cytoplasm, before localising to speckles at the 48 and 72 hour time-points. 3 independent experiments, n=100 cells per experiment. Data shown is mean \pm SD.



Figure S2: NCDN and SNRPB co-localise in neurites of SH-SY5Y cells constitutively expressing mCherry-SNRPB, and transiently expressing NCDN-GFP. Arrows identify SNRPB containing-vesicles with NCDN co-localisation. Bar= 7µm, images are single deconvolved z-sections. Co-localisation images were generated in Volocity, using automatic thresholds on non-deconvolved z-sections (see materials and methods).



Figure S3: Endogenous SNRPB fractionates similarly to YFP-SNRPB and YFP-SNRPN, present in the 100,000 RCF vesicle fraction in and highly enriched in the nuclear pellet of fractionated SH-SY5Y cells. The gap between bands in the whole cell lysate and cell fractions signifies omitted lanes.



Figure S4: Quantified immunoblots of proteins targeted by siRNA in SH-SY5Y cells from Fig 5C. Equal volumes of lysate were immunoblotted, with band intensity of A) GFP-SMN, and B) SMN, NCDN, SNRPB (Y12) and lamin A/C quantified, and normalised to band intensity of tubulin, to determine the relative levels of protein expression.

| | | Molecular | | | |
|------------------------------------------------------------------------------------|------------------|-----------|-------|-------|-----|
| Identified protein | Accession number | Weight | SNRPN | SNRPB | YFP |
| small nuclear ribonucleoprotein polypeptide B * [Homo sapiens] | gi 53690154 | 24 kDa | 13 | 11 | 2 |
| small nuclear ribonucleoprotein Sm D2 isoform 1 [Homo sapiens] | gi 4759158 | 14 kDa | 10 | 8 | 4 |
| small nuclear ribonucleoprotein Sm D1 [Homo sapiens] | gi 5902102 | 13 kDa | 5 | 5 | 4 |
| small nuclear ribonucleoprotein E [Homo sapiens] | gi 4507129 | 11 kDa | 5 | 4 | 2 |
| small nuclear ribonucleoprotein F [Homo sapiens] | gi 4507131 | 10 kDa | 5 | 3 | 0 |
| small nuclear ribonucleoprotein Sm D3 [Homo sapiens] | gi 4759160 | 14 kDa | 4 | 4 | 3 |
| small nuclear ribonucleoprotein G [Homo sapiens] | gi 4507133 | 8 kDa | 4 | 3 | 0 |
| survival motor neuron protein isoform a [Homo sapiens] | gi 13259527 | 30 kDa | 8 | 7 | 0 |
| GEMIN5 protein [Homo sapiens] | gi 219517971 | 168 kDa | 22 | 25 | 0 |
| HC56 (Gemin 4) [Homo sapiens] | gi 10945430 | 119 kDa | 18 | 11 | 0 |
| DEAD box RNA helicase Gemin3 [Homo sapiens] | gi 6503002 | 92 kDa | 12 | 18 | 0 |
| gem-associated protein 2 isoform alpha [Homo sapiens] | gi 4506961 | 32 kDa | 6 | 4 | 0 |
| gem-associated protein 8 [Homo sapiens] | gi 8923481 | 29 kDa | 6 | 2 | 0 |
| Gem (nuclear organelle) associated protein 6 [Homo sapiens] | gi 17390437 | 19 kDa | 3 | 3 | 0 |
| gem-associated protein 7 [Homo sapiens] | gi 13376001 | 15 kDa | 3 | 2 | 0 |
| WD-40 repeat protein (Unrip) [Homo sapiens] | gi 4519417 | 38 kDa | 16 | 16 | 0 |
| U6 snRNA-associated Sm-like protein LSm4 isoform 1 [Homo sapiens] | gi 6912486 | 15 kDa | 5 | 4 | 0 |
| U6 snRNA-associated Sm-like protein LSm2 [Homo sapiens] | gi 10863977 | 11 kDa | 4 | 4 | 0 |
| U6 snRNA-associated Sm-like protein LSm6 [Homo sapiens] | gi 5901998 | 9 kDa | 2 | 2 | 0 |
| U6 snRNA-associated Sm-like protein LSm3 [Homo sapiens] | gi 7657315 | 12 kDa | 2 | 2 | 0 |
| U6 snRNA-associated Sm-like protein LSm7 [Homo sapiens] | gi 7706423 | 12 kDa | 2 | 0 | 0 |
| U7 snRNA-associated Sm-like protein LSm11 [Homo sapiens] | gi 27735089 | 40 kDa | 2 | 0 | 0 |
| protein arginine N-methyltransferase 5 isoform a [Homo sapiens] | gi 20070220 | 73 kDa | 30 | 31 | 3 |
| methylosome protein 50 [Homo sapiens] | gi 13129110 | 37 kDa | 12 | 10 | 0 |
| methylosome subunit pICIn [Homo sapiens] | gi 4502891 | 26 kDa | 7 | 8 | 0 |
| CD2 antigen cytoplasmic tail-binding protein 2 [Homo sapiens] | gi 5174409 | 38 kDa | 13 | 12 | 0 |
| PERQ amino acid-rich with GYF domain-containing protein 2 isoform c [Homo sapiens] | gi 156766047 | 149 kDa | 2 | 4 | 0 |
| WW domain-binding protein 4 [Homo sapiens] | gi 6005948 | 43 kDa | 3 | 0 | 0 |
| formin binding protein 4, isoform CRA_a [Homo sapiens] | gi 119588290 | 90 kDa | 2 | 2 | 0 |
| neurochondrin isoform 2 [Homo sapiens] | gi 62526031 | 79 kDa | 5 | 0 | 0 |
| nuclear receptor coactivator 6 interacting protein, isoform CRA_b [Homo sapiens] | gi 119607168 | 97 kDa | 13 | 0 | 0 |
| 7SK snRNA methylphosphate capping enzyme isoform A [Homo sapiens] | gi 47271406 | 74 kDa | 13 | 0 | 0 |
| RNA-binding protein 40 [Homo sapiens] | gi 40538732 | 59 kDa | 13 | 0 | 0 |

Table S1: A selected dataset from the interactome analysis of SNRPN and SNRPB confirms efficient identification of known Sm protein interactors. All other core Sm family proteins were identified in the interactome analysis, as well as SMN, all Gemin components of the SMN complex and several LSm proteins, including LSm11 (found only in the U7 snRNP). Additionally, several members of the methylosome, where SmB post-translational modifications occur, were identified including Protein arginine N-methyltransferase 5 (PRMT5). Several previously identified SNRPB interactors (CD2 antigen cytoplasmic tail binding protein-2, PERQ2, WW domain-binding protein 4 and Formin binding protein 4) (Bedford et al. 1998, Bedford et al. 2000, Kofler et al. 2004, Kofler et al. 2005) were identified to interact with at least one of the proteins. * denotes that spectra from SNRPN were pooled with those from SNRPB by Scaffold due to sequence similarity in the majority of the proteins. Neurochondrin, and other proteins discussed are also shown here. Values are number of unique peptides identified.



Movie 1: punctate structures containing mCherry-SNRPN within the cytoplasm of SH-SH5Y cells are mobile. Images were taken every ~2 seconds for ~150 seconds. Movie is a projection of 3 deconvolved z-stacks taken with 0.5 μ m spacing. Bar=7 μ m.