Figure S1

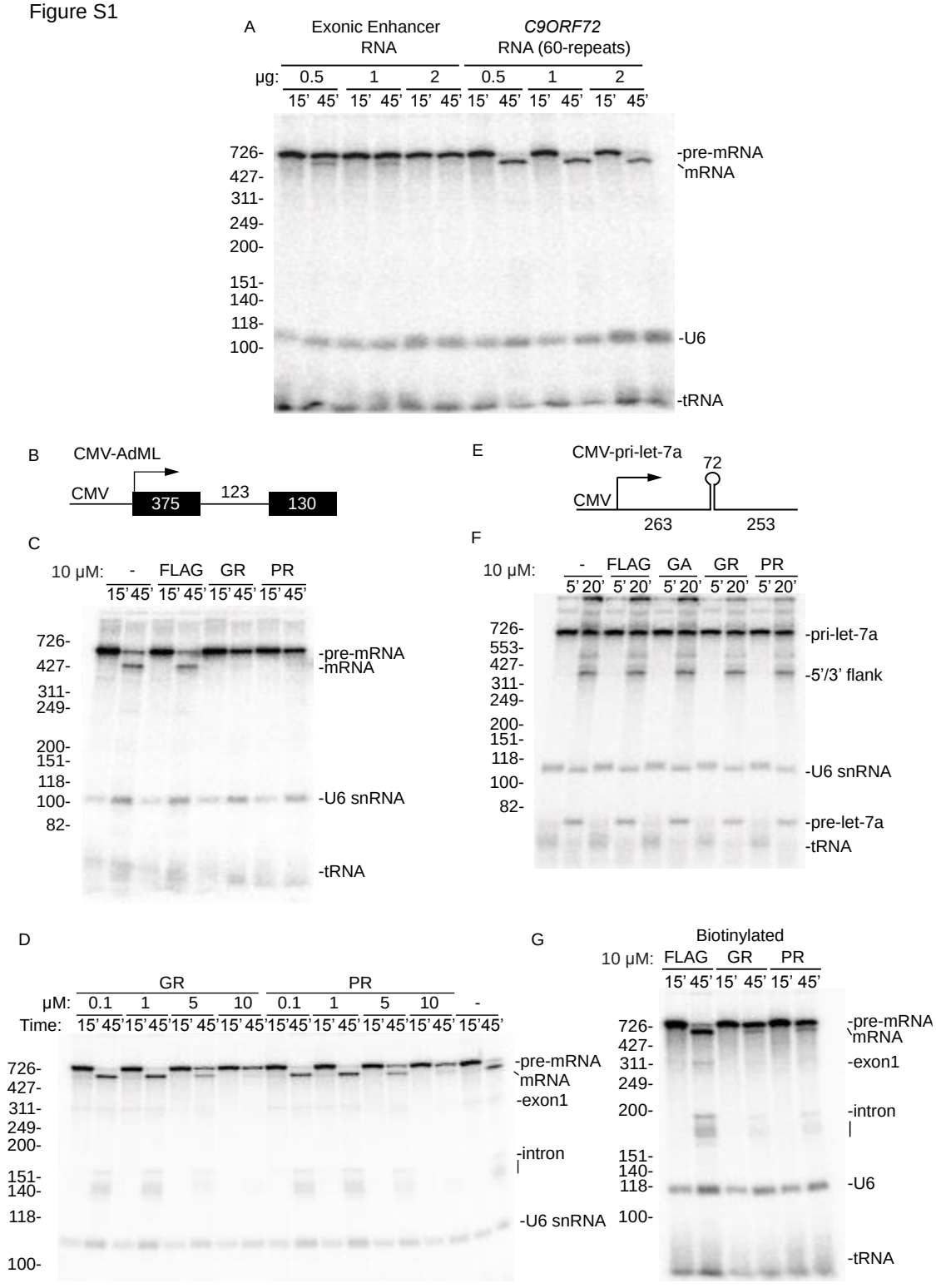


**Figure S1，related to Figures 1 and 2. Effect of *C9ORF72* repeat RNA or DPRs on splicing and microRNA processing.**
(A) *C9ORF72* repeat RNA does not affect splicing in vitro. CMV-Ftz DNA template was incubated in transcription-coupled splicing reaction mixtures for 15 minutes with the indicated RNAs synthesized by T7 polymerase. α-Amanitin was then added to stop transcription, and incubation was continued for 30 minutes. (B) Schematic of CMV-AdML DNA template showing sizes of the intron and exons (in nts). (C) Same as (A) except CMV-AdML was used incubated for 15 minutes in transcription-coupled splicing reaction mixtures containing no peptide or 10 μM of indicate peptides, followed by addition of α-Amanitin and continued incubation for 30 minutes. (D) Same as (C) except that the indicated amounts of peptides were added. (E) Schematic of CMV-pri-let-7a DNA template showing sizes of 5' and 3' flanking regions and primary microRNA. (F) CMV-pri-let-7a DNA was incubated for 5 minutes in no peptide or with 10 μM of indicated peptides, followed by addition of α-Amanitin and incubation for 15 minutes. (G) Same as C except the peptides were biotinylated. In panels D and G, the line below the intron marks intron breakdown products.

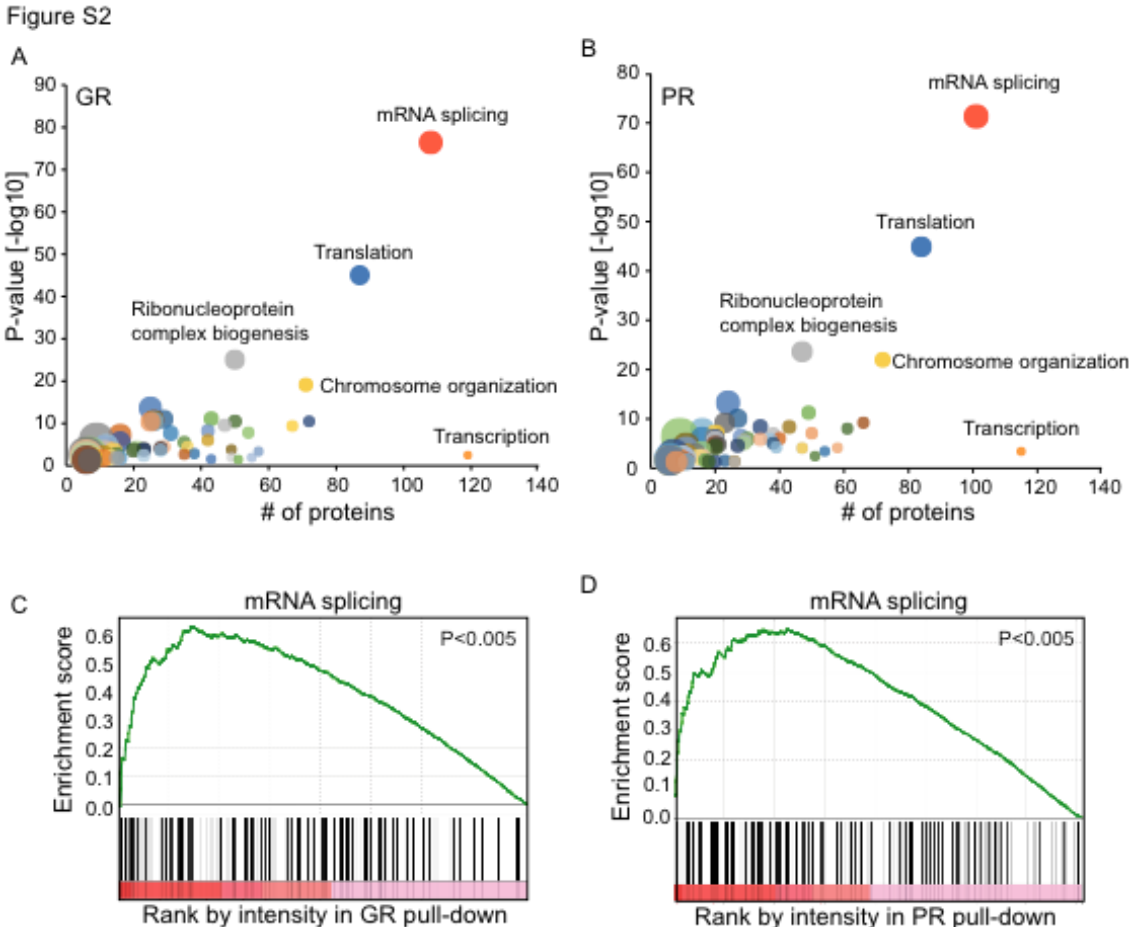


**Figure S2, related to Figure 2. Gene Ontology (GO) analysis and Gene set enrichment analysis (GSEA) for the GR and PR interactomes.** (A, B) GO analysis of interactors detected in GR (A) or PR (B) pulldown. X axis indicates the number of GR interactors that belong to each GO term. Y axis depicts the p-value of enrichment corrected for multiple hypothesis testing using the Benjamini-Hochberg method. The size of the circle indicates the relative amount of GR or PR interactors in each GO term. (C, D) GSEA enrichment profile for splicing proteins in GR (C) or PR (D) pulldown. In every thumbnail, all proteins identified in each pulldown were ranked across the X-axis based on their abundance, with the more abundant proteins to the left side, and black vertical lines indicate the position of proteins involved in splicing. By walking down the list from left to right, the green curve represents the enrichment of splicing proteins. P-value indicates that splicing proteins are the most significantly enriched in the top GR and PR interactors.

Figure S3

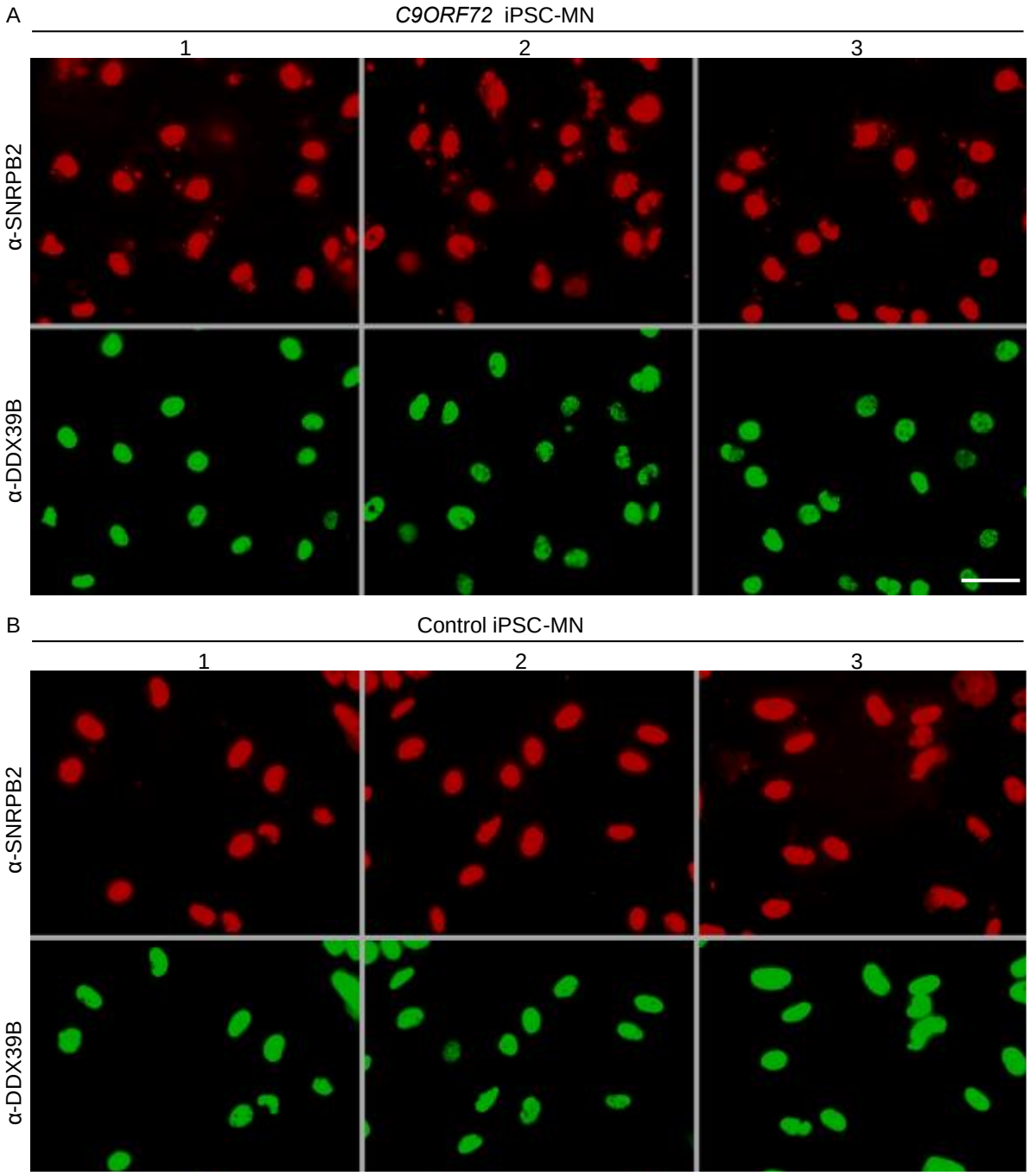


**Figure S3, related to Figure 4. Fields of cells showing SNRPB2 mislocalized to the cytoplasm in *C9ORF72* iPSC-derived motor neurons.** Immunofluorescent staining of iPSC-derived motor neurons from three *C9ORF72* patients or three control subjects was carried out using the indicated antibodies. Scale bar: 20 μm.

Figure S4

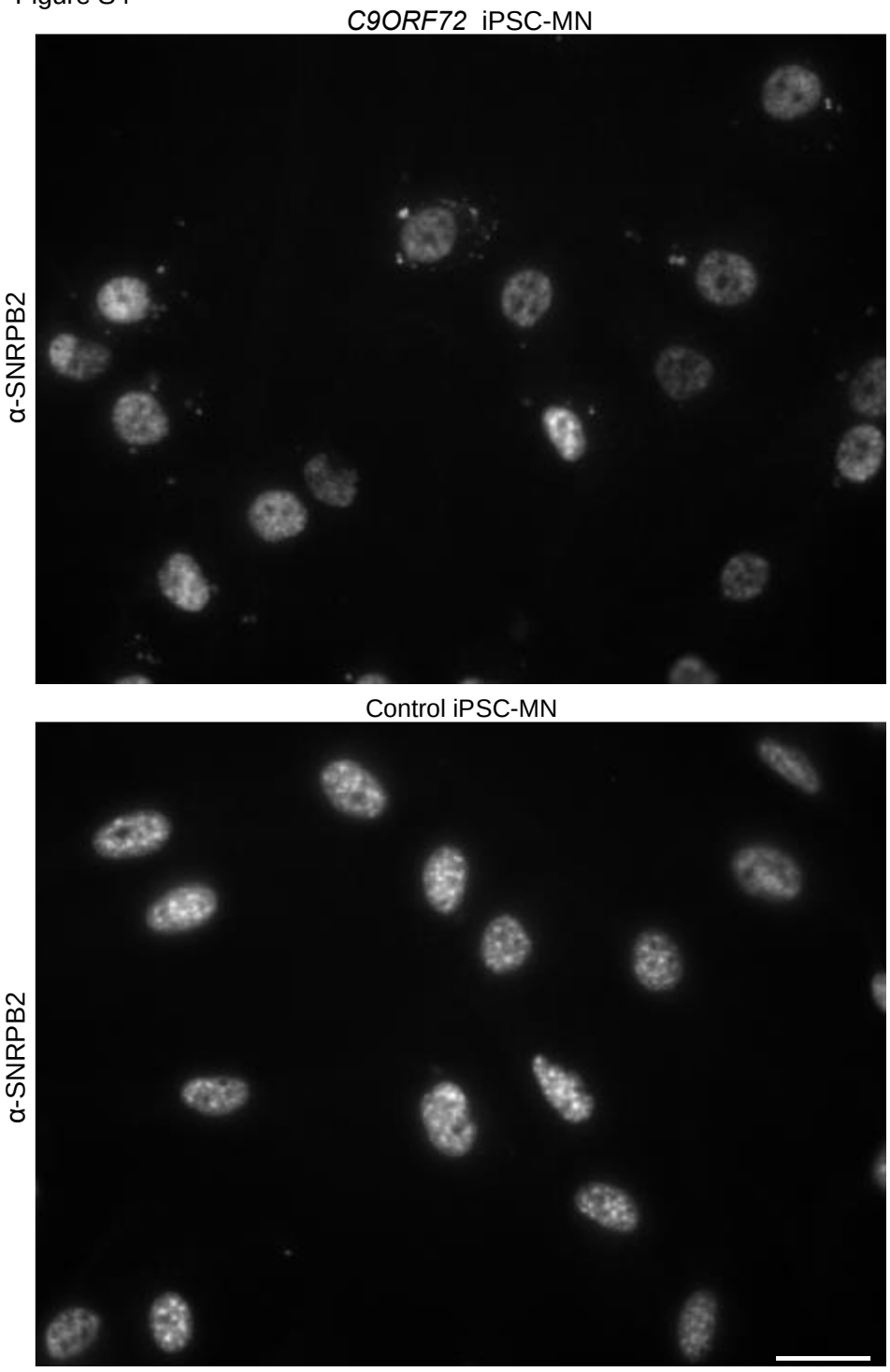


**Figure S4, related to Figure 4. Fields of cells showing SNRPB2 levels are lower in nuclear speckle domains and in the nucleoplasm in *C9ORF72* iPSC-derived motor neurons compared to control.** A short exposure of immunofluorescent staining using the SNRPB2 antibody is shown**.** Three biological replicates were carried out. Scale bars: 20 μm.

Figure S5

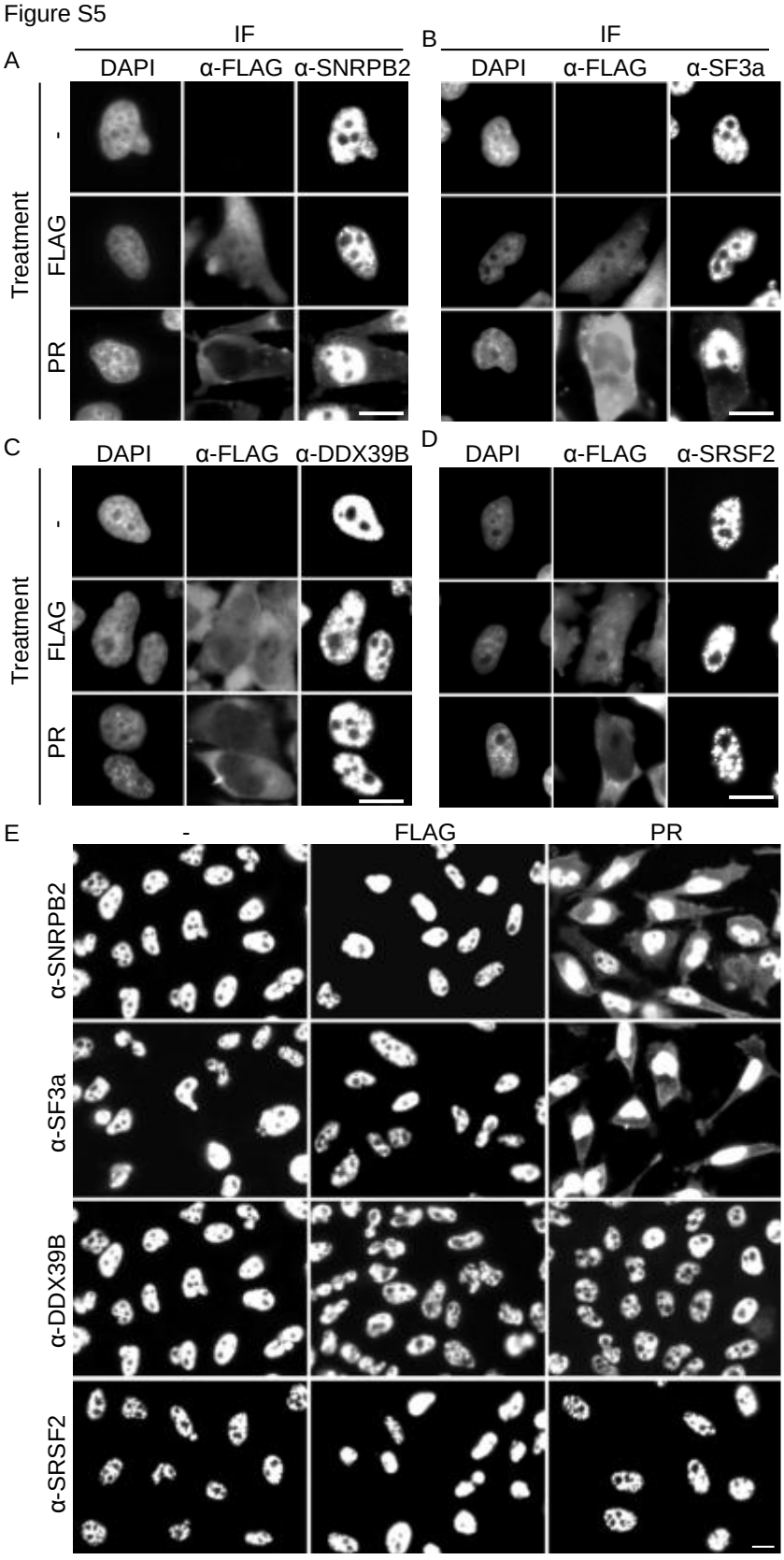


**Figure S5, related to Figure 4. U2 snRNP is mislocalized to the cytoplasm in HeLa cells treated with PR.** (A-D) HeLa cells were treated with no peptide (-), 5 μM FLAG or 5 μM FLAG-tagged PR for 1 hour, and immunofluorescent staining was carried out using antibodies against (A) FLAG and SNRPB2, (B) FLAG and SF3a, (C) FLAG and DDX39B or (D) FLAG and SRSF2. (E) Fields for panels A-D are shown. Scale bars: 20 μm.

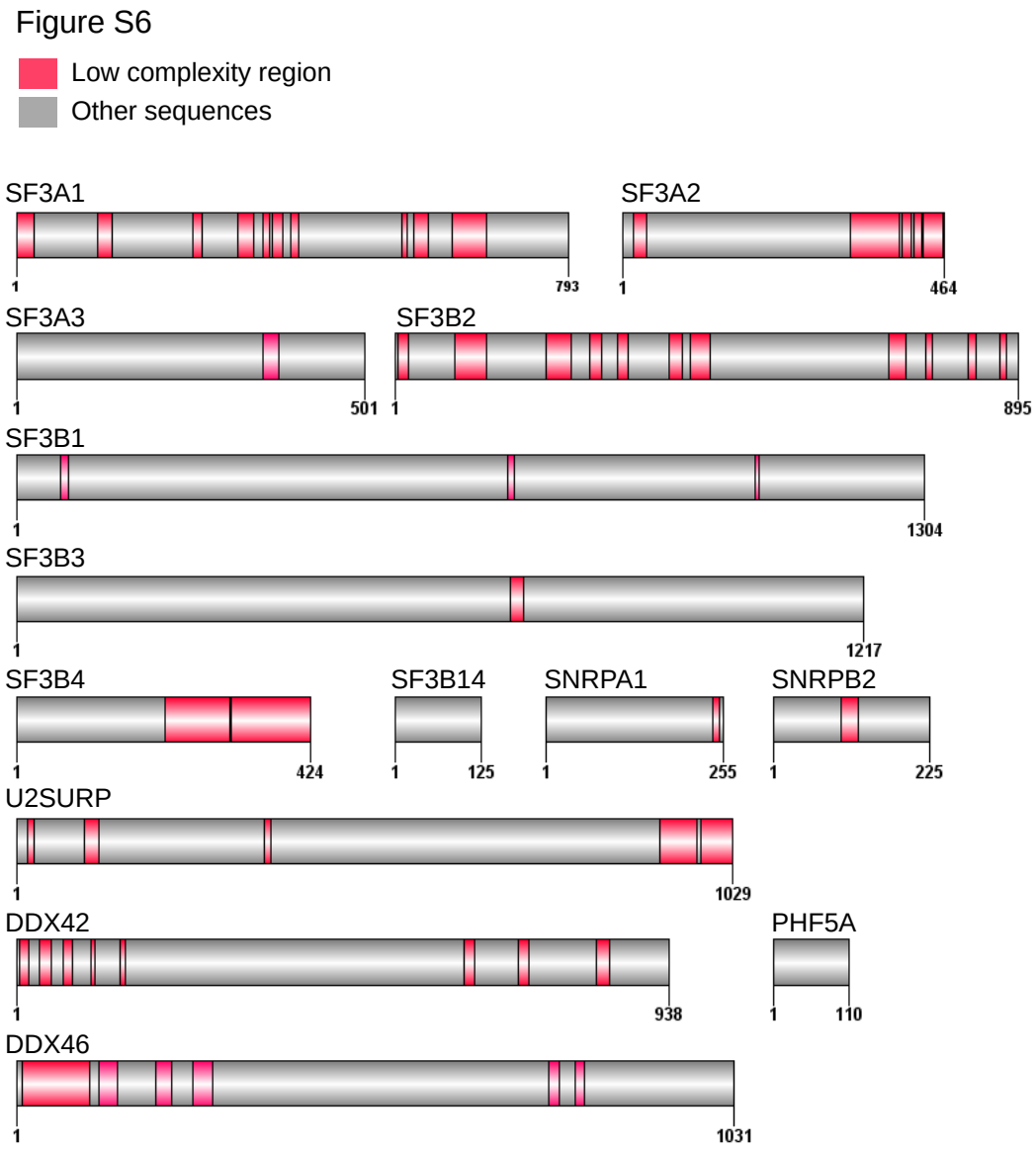


**Figure S6, related to Figure 6. Prediction of low complexity domains (LCDs) in U2 snRNP proteins.** LCDs in U2 snRNP proteins were predicted by SMART software.

**Supplemental Tables**

Table S1, related to Figure 2. Quantitative analysis of proteins associated with GR, PR and FLAG.
Table S2, related to Figure 2. Gene Ontology of proteins associated with GR.
Table S3, related to Figure 2. Gene Ontology of proteins associated with PR.
Table S4, related to Figure 5. U2-dependent genes in top 200 mis-spliced exons in *C9ORF72* patient cerebellums.
Table S5, related to Figure 5. U2-dependent genes identified in the 107 mis-spliced exons in *C9ORF72* patient frontal cortex.
Table S6, related to Figure 5. U2-dependent genes that are also PR dependent in *C9ORF72* cerebellum and frontal cortex in Tables S4 and S5.

**Supplemental Experimental Procedures**

**Plasmids**

The positive control plasmid encoding the Exonic Enhancer RNA was described (Das et al., 2007). The plasmid encoding 60 copies of the *C9ORF72* repeats was a generous gift from Dr. J. Paul Taylor. Both plasmids were cut with BamHI and transcribed with T7 polymerase to generate RNAs. CMV-AdML DNA template and CMV-pri-let-7a DNA template were described in (Das et al., 2006) and in (Yin et al., 2015), respectively. CMV-DNA templates were amplified by PCR using forward (5'-TGGAGGTCGCTGAGTAGTGC-3') and reverse (5'- TAGAAGGCACAGTCGAG GCT-3') primers, and used in transcription-coupled splicing or transcription-coupled pri-miRNA processing reaction mixtures.

**Transcription-coupled RNA processing**

For transcription-coupled splicing, Ftz or AdML DNA template (200 ng) was assembled into pre-initiation complexes (PICs) by incubation in 15 µl HeLa nuclear extract, 3.2 mM $MgCl_2$ and 5 µl polyvinyl alcohol for 20 minutes at 30°C (Yu et al., 2010). Subsequently, the indicated amounts of peptides or RNAs were added and incubation was continued for the times indicated to allow transcription in the presence of 0.5 mM ATP, 20 mM creatine phosphate (di-Tris salt), and 1 µl $^{32}$P-UTP (250 Ci/mmol; Perkin Elmer Life Sciences) in a final reaction mixture volume of 25 µl. α-Amanitin (200 ng) was added to stop transcription, and incubation was continued at 30°C for times indicated. For transcription/splicing/pri-miRNA processing, CMV-pri-let-7a DNA was assembled into a PIC using the conditions for transcription-coupled splicing. The indicated amounts of peptides were then added and incubation was continued for 5 minutes to allow transcription in the presence of 3.2 mM $MgCl_2$, (final $MgCl_2$ in the reaction is 6.4 mM), 0.5 mM ATP, 20 mM creatine phosphate (di-Tris salt), and 1 µl $^{32}$P-UTP (250 Ci/mmol; Perkin Elmer Life Sciences) in a final reaction mixture volume of 25 µl. After adding α-Amanitin (200 ng), incubation was continued for 15 minutes to allow processing. Total RNA was fractionated on 8% denaturing polyacrylamide gels and detected by PhosphorImager.

**Bioinformatic analysis**

Gene Ontology (GO) analysis of the significant interactors of GR or PR (>2 fold than in FLAG pulldown) was performed using DAVID functional annotation tool (https://david.ncifcrf.gov/). Gene Set Enrichment Analysis (GSEA) analyses of these interactors were performed using http://software.broadinstitute.org/gsea/index.jsp. GSEA analysis ranked all the identified proteins across the x-axis based on their abundance, with the more abundant proteins identified in the pulldown to the left end. The position of the proteins involved in mRNA splicing was labelled as black vertical lines. Their ranks were used to determine the enrichment score (ES) and the p-value, which reflect the degree to which a gene set is overrepresented at the top or bottom of a ranked list of genes. LCDs in U2 snRNP proteins were predicted by SMART (Simple Modular Architecture Research Tool) software (Letunic et al., 2015), and the images were prepared with DOG 2.0 software.

**Immunofluorescent (IF) staining**

For IF staining of iPSC-motor neurons (MNs), 3 patient and 3 controls were fixed with 4% paraformaldehyde in PBS for 15 minutes and permeablized with 0.1% Triton X-100 in PBS for 15 minutes. Cells were then incubated overnight at 4° in the indicated antibodies followed by 3 washes in PBS and incubation in secondary antibody for 1 hour at room temperature. After 3 washed in PBS, IF staining was carried out. Fields of cells were captured with a Nikon TE2000U inverted microscope (We note that the same methods were used to obtain the larger magnification images shown in main text Figure 4). In Figure S4, *C9ORF72* patient 3 and control 2 were used.

For IF staining of HeLa cells, no peptide, 5 µM FLAG or 5 µM FLAG-tagged PR were incubated for 1 hour at 37°. Cells were fixed with 4% paraformaldehyde in PBS for 15 minutes and permeabilized with 0.1% Triton X-100 in PBS for 15 minutes. After incubation in 5% FBS for 1 hour at room temperature, cells were incubated overnight at 4°C in the primary antibodies SNRPB2 (1:100), DDX39B (1:1000), SF3a (1:1000) and SRSF2 (1:1000). Three washes in PBS were then carried out, and cells were incubated in a 1:1000 dilution of mouse Alexa-647 (for SNRPB2 and SRSF2) or rabbit Alexa-488 secondary (for SF3a and DDX39B) antibodies for 1 hour at room temperature. This was followed by 3 washes in PBS and images were captured with a Nikon TE2000U inverted microscope.