Supplemental Information

Extended Experimental Procedures

Plasmids

GST-FUS and truncations were constructed by inserting a PCR fragment containing full-length or portions of FUS into the BamHI and XhoI sites of pGEX-6P-1. Primer sequences are listed in Table S2. N-terminal 3 x FLAG tagged FUS full-length and R495X were constructed by inserting PCR fragments into the BamHI and XhoI sites of pcDNA5/FRT/TO (Invitrogen) containing 3 x FLAG tag at the Hind III site.

RNAi

Lentivirus-mediated shRNA was used against FUS (CCGGCGTGGTGGCTTCAATAAATTTCTCGAGAAATTTATTGAAGCCACCACGTTTTT, Open Biosystems) with a scrambled negative control (CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG, Addgene). FUS siRNA, TDP-43 siRNA (ON-TARGET SMARTpool) and non-targeting control were from Thermo Scientific.

Immunoprecipitation (IP) and GST pulldowns

For IPs, antibodies were coupled to protein A (polyclonal) or G (monoclonal) and covalently crosslinked using dimethylpimelimidate (Sigma). A 500 μl mixture containing 150 μl of pre-cleared HeLa nuclear extract, 150 μl splicing dilution buffer (20 mM Hepes pH7.9, 100 mM KCl), 500 μM ATP, 3.2 mM $MgCl_2$ and 20 mM creatine phosphate was incubated for 30 min at 30°C. RNase A was included at a

concentration of 50 ng/μl for +RNase conditions. After incubation, mixtures were spun at 4°C for 5 min at 14k rpm. Supernatants were added to 250 μl of IP buffer (1× PBS, 0.1% Triton-X100, 0.2 mM PMSF, protease inhibitor EDTA-free (Roche)), spun at 4°C for 5 min, and added to 40μl antibody- crosslinked beads. After rotation overnight at 4°C, 6 washes (1.5 ml each) were done using wash buffer (1X PBS containing 0.1% Triton-X100, 0.2 mM PMSF). Proteins were eluted by adding 60 μL SDS sample loading buffer, followed by incubation for 20 min at RT. DTT was added to a final concentration of 5 mM, samples were boiled for 4 min, and loaded on a 4%–12% SDS gradient gel. For RNA IPs, total RNA was isolated, and analyzed on 5% denaturing gels stained with ethidium bromide. GST pulldowns from nuclear extract were carried out in the same mixtures used for IPs. GST (2 μg) or GST-fusion proteins (2 μg) and 10 μl glutathione beads were added to GST-pre-cleared extract. Pulldowns were rotated at 4°C overnight and washed five times with wash buffer. Proteins were eluted the same as for IPs. For GST-pulldowns using purified proteins, mixtures containing 2 μg of GST-fusion protein, 2 μg of His-fusion protein, 50 ng/μl RNase A and 10 μl of glutathione sepharose 4B were rotated overnight at 4°C. Pulldowns were washed 5 times with wash buffer.

Supplemental Figure legends

Supplemental Figure S1, related to Figure 1. FUS and U1 snRNP are specifically associated with FUS and the RRM motif of FUS is required for binding U1 snRNP and the SMN complex. (**A, B**) Characterization of FUS rabbit polyclonal antibody. The FUS antibody detects one main band by Western (**A**) and immunoprecipitates one main band (**B**). We note that FUS migrates at different positions on SDS versus SDS gradient gels. Molecular weight markers (in kD) are indicated. (**C, D**) The FUS antibody does not immunoprecipitate U1 snRNP in FUS knockdown nuclear extract. (**C**) U1-70K, U1A, SmB/B' and SmD co-IP with FUS in sh control knockdown nuclear extract but not in sh FUS knockdown nuclear extract. (**D**) Same as C, except showing that U1 snRNA does not co-IP with FUS in FUS knockdown nuclear extract. Control antibodies in lanes 2 and 8 are mouse IgG and in lanes 6 and 12 are non-related rabbit serum. (**E**) Schematic of FUS showing domain organization. Schematics of truncated FUS proteins are shown below. Black shading indicates the FUS truncation containing the RRM domain, which is required for FUS binding to U1 snRNP and the SMN complex. (**F**) Coomassie-stained gel (upper image) showing GST pulldowns from buffer (-) or nuclear extract (NE) (+) using GST alone, full length (FL) GST-FUS, or the indicated GST-FUS truncated proteins. * indicates FUS. Molecular weight markers in kD are indicated. Western blots with the indicated antibodies using a portion of the samples is shown below. (**G**) Ethidium bromide-stained gel showing total RNA isolated after GST pulldowns using the indicated FUS truncated proteins. The snRNAs and tRNA are indicated. (**H**) Coomassie gel showing GST pulldowns from buffer (-) or nuclear extract (NE) (+)

using GST alone, full length (FL) GST-FUS, or the indicated GST-FUS truncated proteins. * indicates FUS. Molecular weight markers in kD are indicated.

Supplemental Figure S2, related to Figure 2. SMN and Gemin3 co-localize in Gems in HeLa cells. IF was performed with a rabbit polyclonal antibody against SMN and a mouse monoclonal antibody against Gemin3. DAPI was used to stain the nucleus. The merged images are shown.

Supplemental Figure S3, related to Figure 3. The NLS of FUS is not required for direct interactions with SMN. A. Coomassie-stained gel showing GST pulldowns from buffer (-) or nuclear extract (NE) (+) using GST alone, full length (FL) GST-FUS, or GST-FUS ΔNLS. Molecular weight markers in kD are indicated. B. Western blots with the indicated antibodies using a portion of the samples in panel A.

Supplemental Figure S4, related to Figure 4. Characterization of human ALS patient fibroblasts and analysis of Gem levels in TDP-43 knockdown HeLa cells. A. Gems in normal human fibroblasts were detected by co-staining with a mouse monoclonal (2B1) and rabbit polyclonal (H-195) antibody against SMN. DAPI and merged images are shown. **B**. FUS is mislocalized to the cytoplasm in ALS patient fibroblasts containing mutations in the FUS NLS. The indicated ALS patient or unaffected fibroblasts were stained with FUS antibodies. **C**. TDP-43 is localized in the nucleus in both normal and ALS patient fibroblasts containing TDP-43 mutations. The indicated ALS patient or unaffected fibroblasts were stained with TDP-43 antibodies.

C. Knockdown of TDP-43 results in loss of Gems in HeLa cells. HeLa cells were transfected with siRNA against TDP-43 or non-targeting negative control siRNA. Cells were stained with TDP-43 or SMN and DAPI was used to detect the nucleus. The TDP-43 staining shows that TDP-43 was efficiently knocked down and the SMN staining shows that the level of Gems is significantly reduced.

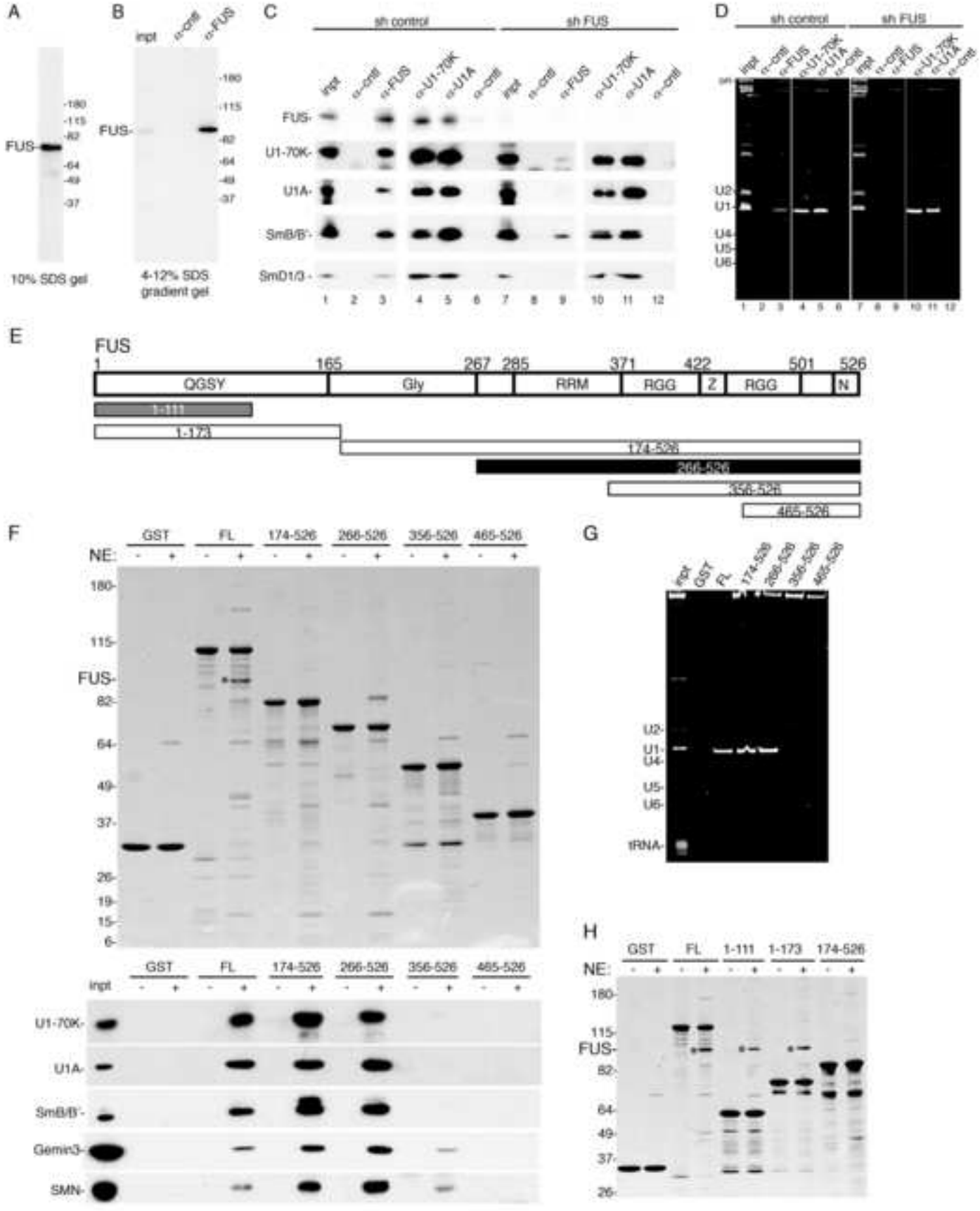
Supplemental Table S1, Proteins present in FUS IP, related to Figure 1 (See attached Excel file)

Supplemental Table S2

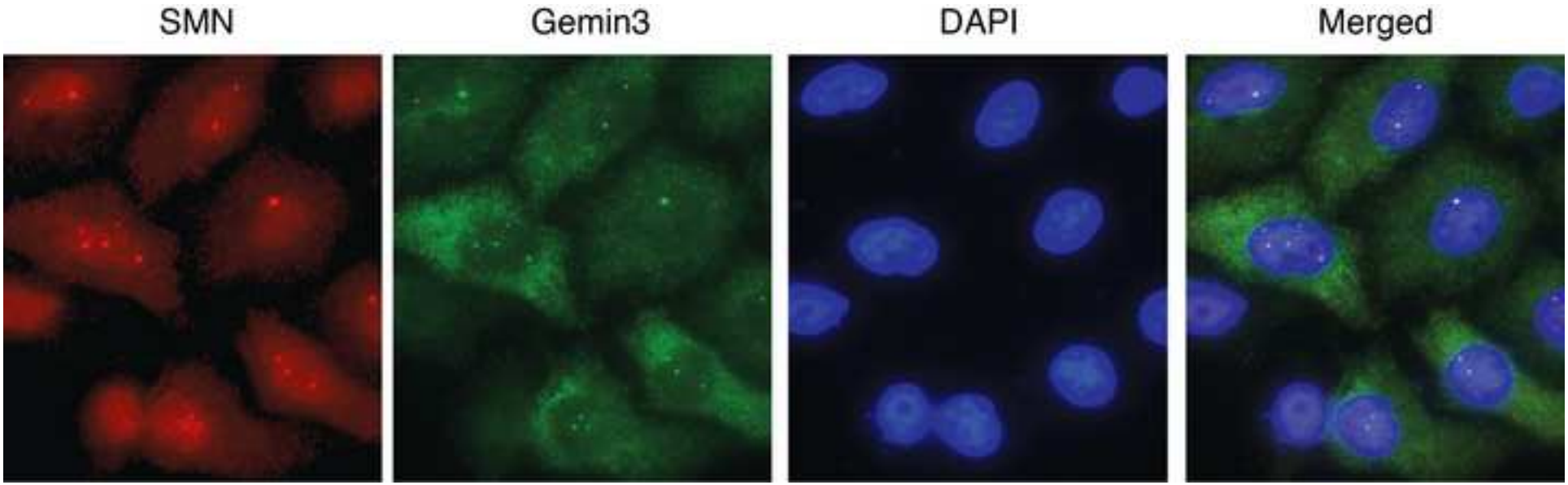
Primers used to construct GST-FUS deletions, related to Experimental Procedures

Primer name	Primer sequence
GST-FUS-1-111 forward	5'-ACGGGATCCATGGCCTCAAACGATTATAC-3'
GST-FUS-1-111 reverse	5'-TCCGCTCGAGTTATCCCGAGGTGCTGCTGGGAG-3'
GST-FUS-1-173 forward	5'-ACGGGATCCATGGCCTCAAACGATTATAC-3'
GST-FUS-1-173 reverse	5'-TCCGCTCGAGTTAACCTCCACCTCCACCTCCA-3'
GST-FUS-174-526 forward	5'-ACGGGATCCGGAGGTAACTATGGCCAAGA-3'
GST-FUS-174-526 reverse	5'-CAAGACCCGTTTAGAGGC-3'
GST-FUS-266-526 forward	5'-ACGGGATCCGAGGTGGCCCTCGGGACCAAG-3'
GST-FUS-266-526 reverse	5'-CAAGACCCGTTTAGAGGC-3'
GST-FUS-356-526 forward	5'-ACGGGATCCGGTAAAGAATTCTCCGGAAATC-3'
GST-FUS-356-526 reverse	5'-CAAGACCCGTTTAGAGGC-3'
GST-FUS-465-526 forward	5'-ACGGGATCCGGGGGTAACTACGGGGATGA-3'
GST-FUS-465-526 reverse	5'-CAAGACCCGTTTAGAGGC-3'
GST-FUS-ΔNLS forward	5'-ACGGGATCCATGGCCTCAAACGATTATAC-3'
GST-FUS-ΔNLS reverse	5'-TCCGCTCGAGTTAGGAATCCATCTTGCCAGGGC-3'

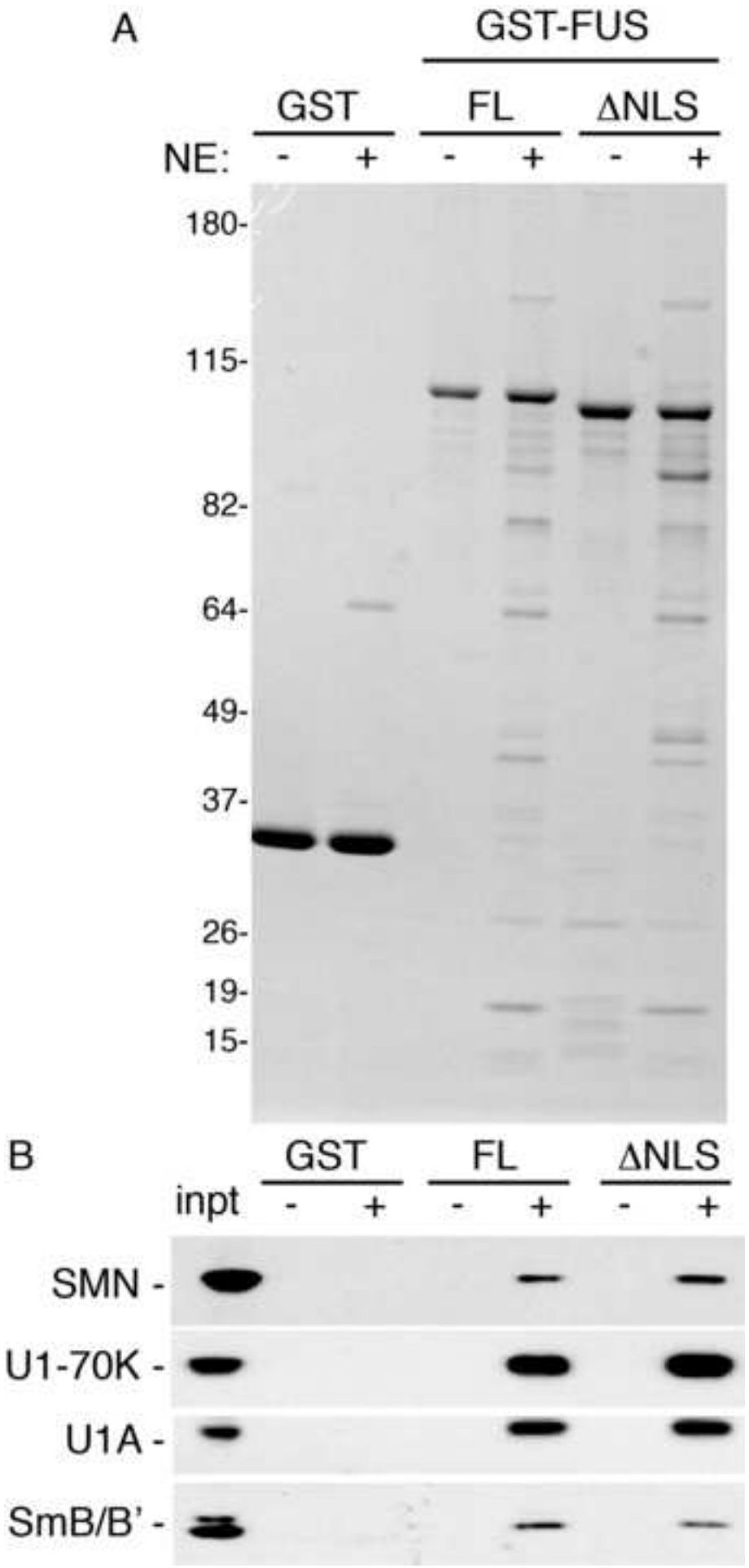
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4