Supplemental Data

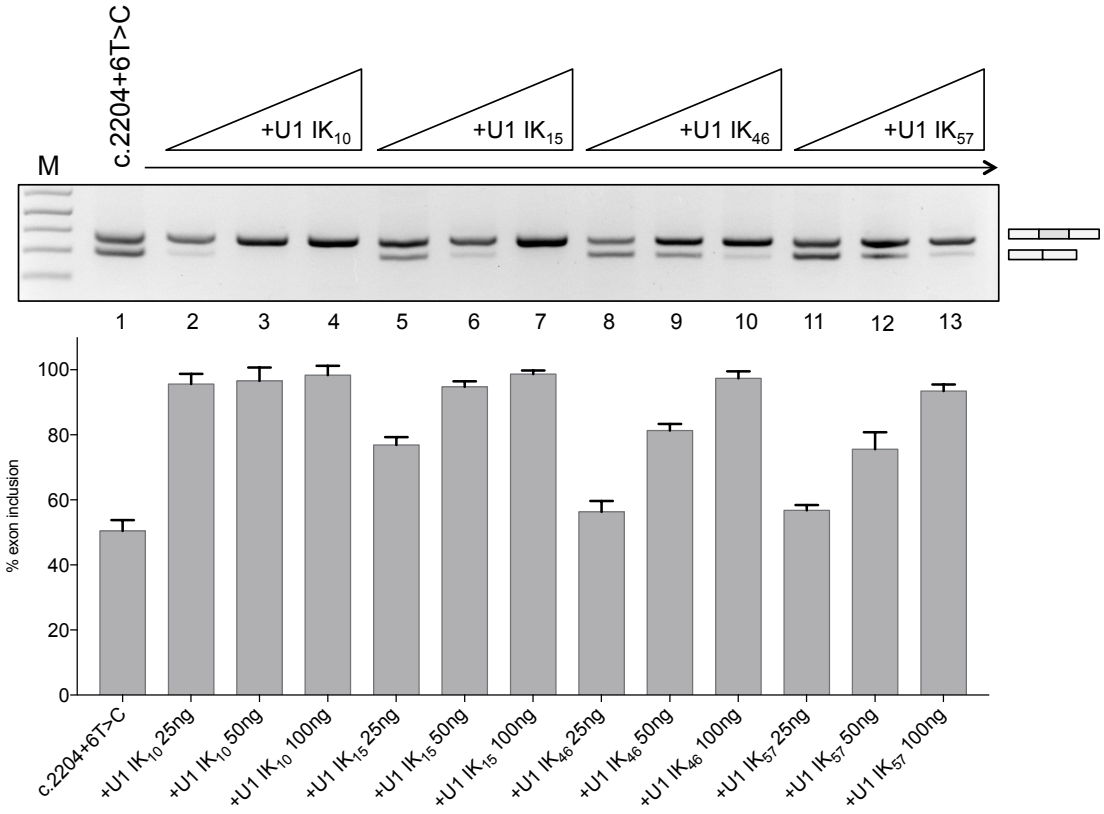

Figure S1. Dose-dependence effect of ExSpeU1s on *ELP1* minigene.

Fixed amount (500ng) of *ELP1* minigene was co-transfected with increasing concentrations (25ng, 50ng and 100ng) of the indicated ELP1-ExSpeU1s (Ik10, Ik15, Ik46 and Ik57) in Hek293T cells. The upper band of 349 bp corresponds to transcripts including the exon 20; the lower band of 275 bp to exon 20 skipping. Mutant *ELP1* (C.2204+6T>C) and ExSpeU1s (Ik10-57) are indicated. A schematic representation of the splicing pattern is on the right of the RT-PCR gel analysis. The graph represents the percentage of exon 20 inclusion and data are expressed as mean ± SD of three independent experiments.

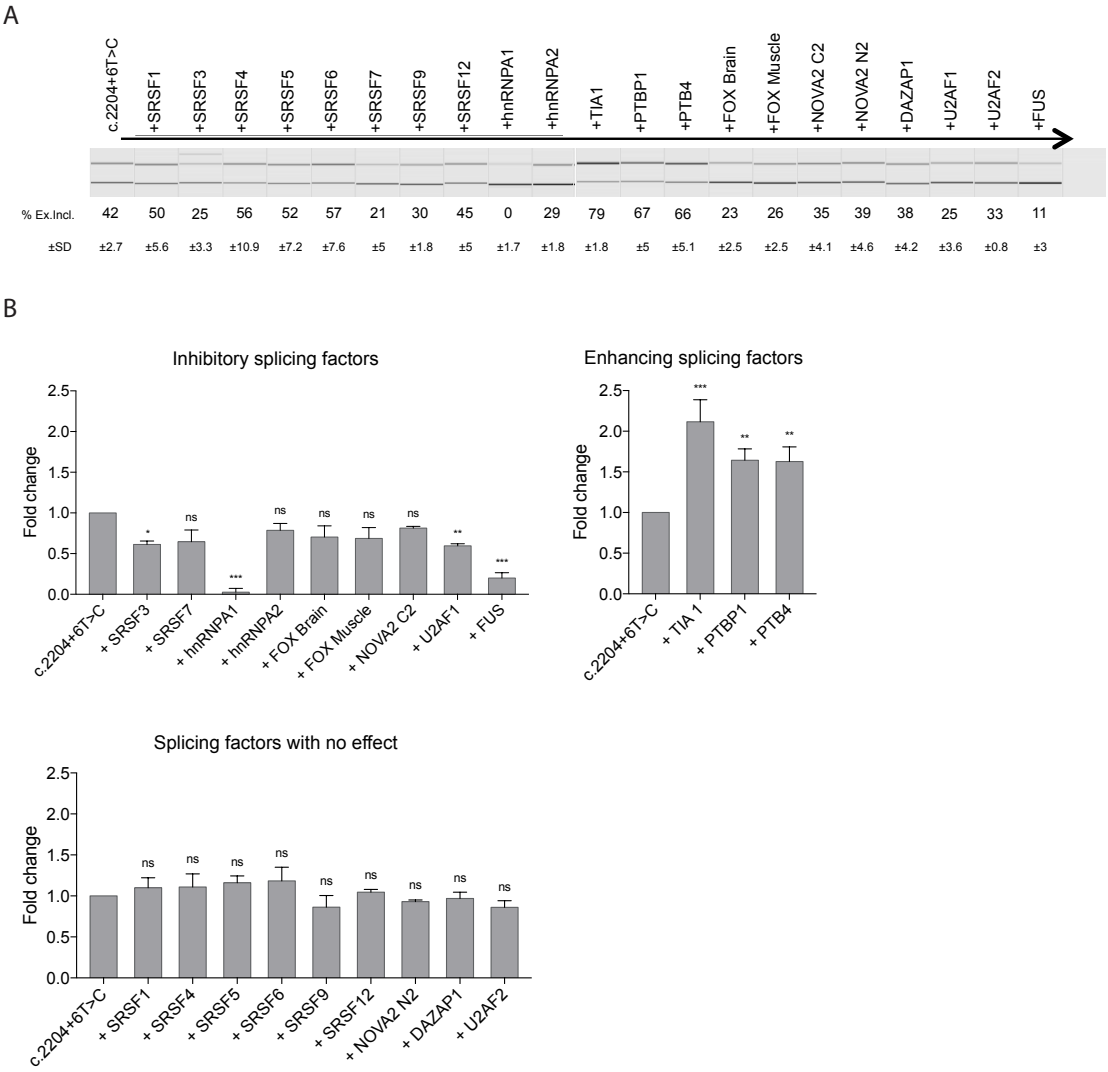

Figure S2. Effects of splicing factors' overexpression in the *ELP1* minigene.

A) Co-transfection experiment of mutant *ELP1* minigene with plasmids overexpressing a panel of splicing factors (indicated at the top) in Hek293T cells. PCR products were analyzed with QIAxcel automated DNA electrophoresis. The calculated percentage of exon 20 inclusion is indicated below.

B) Analysis of the fold splicing changes induced by splicing factors on the c.2204+6T>C *ELP1* minigene. The graphs show splicing factors with inhibitory, enhancing and no effects. Transfection of the mutated *ELP1* minigene alone is set to one. Data are expressed as mean ± SD of three independent experiments. Statistical

analysis was performed using a two-ways ANOVA (***p<0,0001, **p<0,001, *p<0,01; ns, not significant).

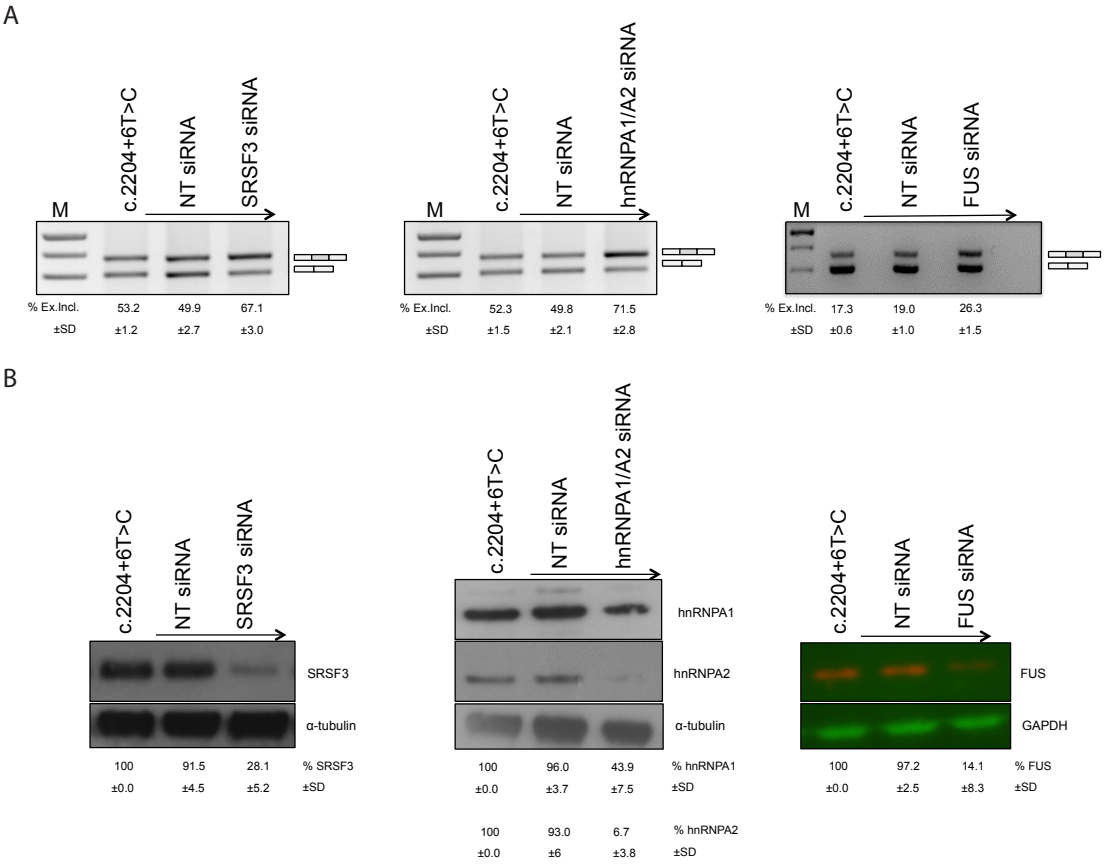

Figure S3. Silencing of inhibitory splicing factors improve *ELP1* exon 20 splicing. Silencing of SRSF3, hnRNP A1/A2 and FUS in Hek293T cells transfected with *ELP1* minigenes. NT was a scrambled siRNA used as negative control. A) Semi-quantitative PCR of the *ELP1* splicing pattern. The upper band of 349 bp corresponds to transcripts including the exon 20; the lower band of 275 bp corresponds to exon 20 skipping. PCR products were resolved on a 2% agarose gel and the intensity of bands was quantified with ImageJ software. The percentage of exon 20 inclusion is indicated below as mean ± SD of a triplicate experiment.

B) Western Blot analysis of the silenced proteins, which are indicated in the upper part. α-tubulin and GAPDH were used as reference for internal normalization.

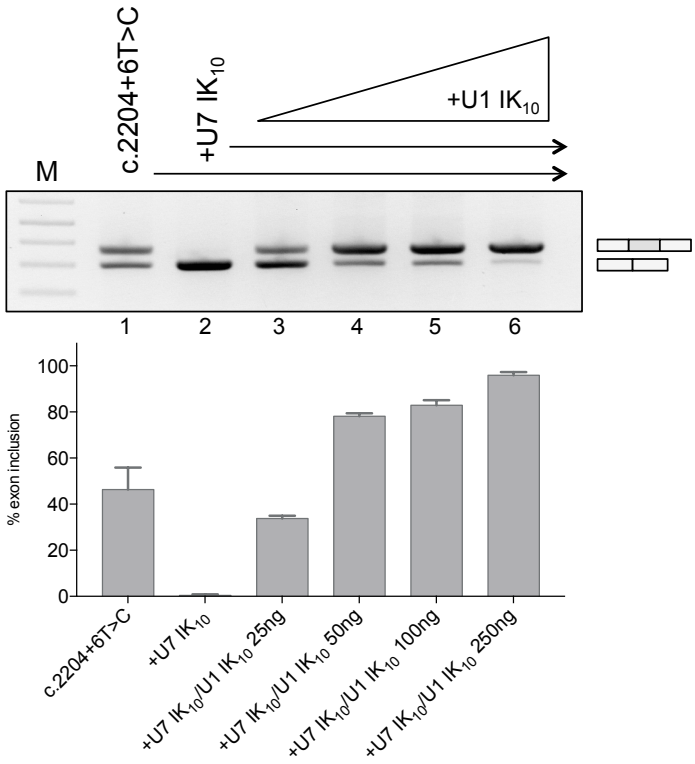

Figure S4. Effects of competition between U7-Ik10 and ExSpeU1 Ik10 particles.

ELP1 mutant minigene was co-transfected with a fixed amount (500ng) of U7-Ik10 minigene and increasing concentrations (25ng, 50ng, 100ng and 250ng) of ExSpeU1 Ik10. The upper band of 349 bp corresponds to transcripts including the exon 20; the lower band of 275 bp to exon 20 skipping. Mutant *ELP1* (C.2204+6T>C), U7-Ik10 and ExSpeU1 Ik10 are indicated. A schematic representation of the splicing pattern is on the right of the RT-PCR gel analysis. The graph represents the percentage of exon 20 inclusion and data are expressed as mean ± SD of three independent experiments.

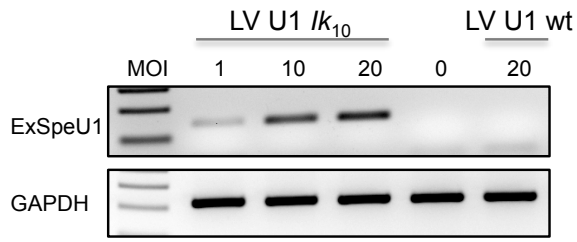

Figure S5. ExSpeU1 Ik10 expression in FD patients' fibroblasts after lentiviral transduction.

In the upper part, Endpoint RT-PCR of the ExSpeU1 Ik10 in FD fibroblasts untreated and transduced with increasing concentrations (MOI) of lentiviral particles expressing the ExSpeU1 Ik10 (LV U1 Ik10) and lentiviral particles expressing the wild-type U1 snRNA (LV U1 wt). In the lower part, Endpoint RT-PCR of GAPDH used as reference for internal normalization.

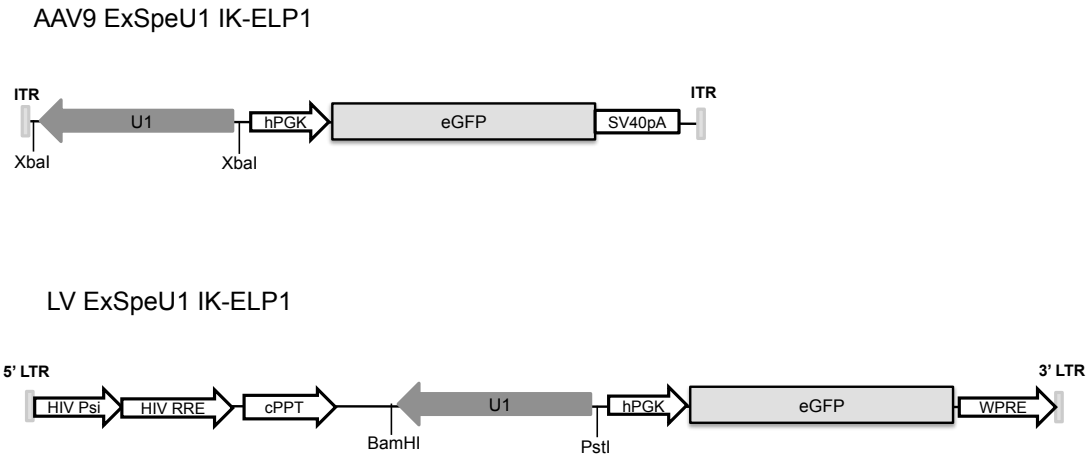

Figure S6. Lentiviral and adeno-associated viral backbones' structures.

Schematic representation of the ExSpeU1 cloning site within the adeno-associated virus backbone (AAV9-ExSpeU1 Ik-ELP1) and within the lentiviral backbone (LV-ExSpeU1 Ik-ELP1).

Primers' name	sequence 5' -> 3'
U1 Ik4 F	GATCTCATAAATGGCGCTGCAGGGGAGATACCAT
U1 Ik4 R	GATCATGGTATCTCCCCTGCAGCGCCATTTATGA
U1 Ik10 F	GATCTCATAGCAAACAGTACAATGCAGGGGAGATACCAT
U1 Ik10 R	GATCATGGTATCTCCCCTGCATTGTACTGTTTGCTATGA
U1 Ik15 F	GATCTCATAGCAAACAGTGCAGGGGAGATACCAT
U1 Ik15 R	GATCATGGTATCTCCCCTGCACTGTTTGCTATGA
U1 Ik34 F	GATCTCATACTTGTGATTTATGCAGGGGAGATACCAT
U1 Ik34 R	GATCATGGTATCTCCCCTGCATAAATCACAAGTATGA
U1 Ik46 F	GATCTCATAGTGTGAAGACAATGCAGGGGAGATACCAT
U1 Ik46 R	GATCATGGTATCTCCCCTGCATTGTCTTCACACTATGA
U1 Ik57 F	GATCTCATAATAAGTATTTTATGCAGGGGAGATACCAT
U1 Ik57 R	GATCATGGTATCTCCCCTGCATAAAATACTTATTATGA
U1 Ik72 F	GATCTCATACAATTTCGAGGCAGGGGAGATACCAT
U1 Ik72 R	GATCATGGTATCTCCCCTGCCTCGAAATTGTATGA
U1 Ik77 F	GATCTCATATCGAGAACTTATGCAGGGGAGATACCAT
U1 Ik77 R	GATCATGGTATCTCCCCTGCATAAGTTCTCGATATGA
U1 Ik99 F	GATCTCATAAGCCCTCATTACGCAGGGGAGATACCAT
U1 Ik99 R	GATCATGGTATCTCCCCTGCGTAATGAGGGCTTATGA
U1 Ik123 F	GATCTCATATCAGATTCTTAGGCAGGGGAGATACCAT
U1 Ik123 R	GATCATGGTATCTCCCCTGCCTAAGAATCTGATATGA
U7 Ik10 F	ACAGAGGCCTTTCCGCATCGCAAACAGTACAATAATTTTTGGAG
SP6 R	ATTTAGGTGACACTATAGAA
alpha 2,3 F	CAACTTCAAGCTCCTAAGCCACTG
ELP1 Ex19 F	GGCCGGCCTGAGCAGCAATCATGTGTCC
ELP1 Ex21 R	GATTCTCAGCTTTCTCATGCATTC
ELP1 FL F	GCAGCAATCATGTGTCCCA
ELP1 FL R	ACCAGGGCTCGATGATGAA
ELP1 Δ20 F	CACAAAGCTTGTATTACAGACT
ELP1 Δ20 R	GAAGGTTTCCACATTTCCAAG
U1 Ik10 tr F	ATAGCAAACAGTACAATGC
U1 Ik10 tr R	CACTACCACAAATTATGCA
Tot ELP1 F	GCTGTTCCCACACCCTGT

Tot ELP1 R	AGGGTCAGCACTTGGACAA
mGAPDH F	ATGGTGAAGGTCGGTGTGAA
mGAPDH R	GTTGATGGCAACAATCTCCA
hGAPDH F	GACAGTCAGCCGCATCTTCT
hGAPDH R	TTAAAAGCAGCCCTGGTGAC
siRNA SRSF3	GAGUGGAACUGUCGAAUGG
siRNA hnRNP A1	CAGCUGAGGAAGCUCUUCA
siRNA hnRNP A2	GGAACAGUUCCGUAAGCUC
siRNA NT	UAAGGCUAUGAAGAGAUAC

Table S1. Sequences of DNA oligonucleotides used for semi-quantitative, quantitative analysis and siRNAs.