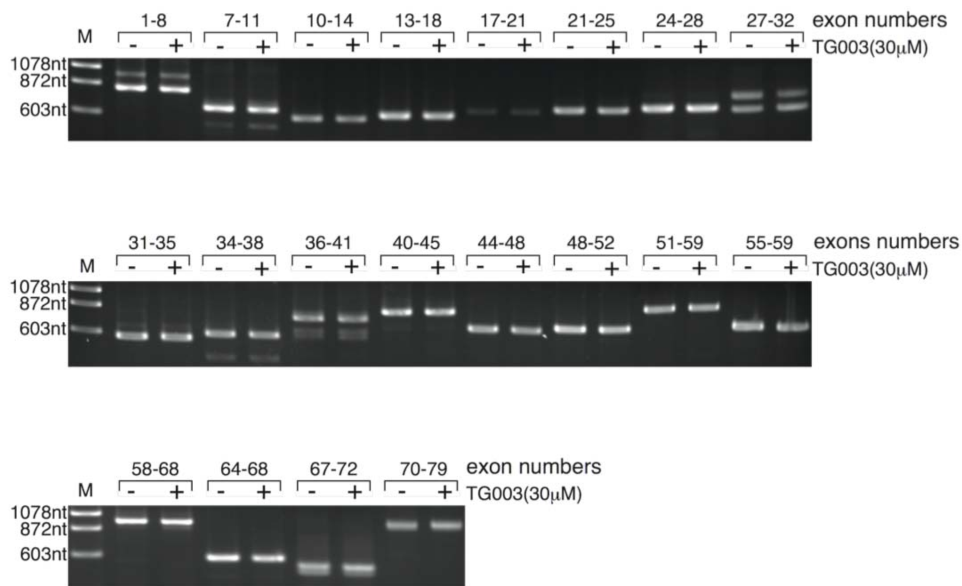
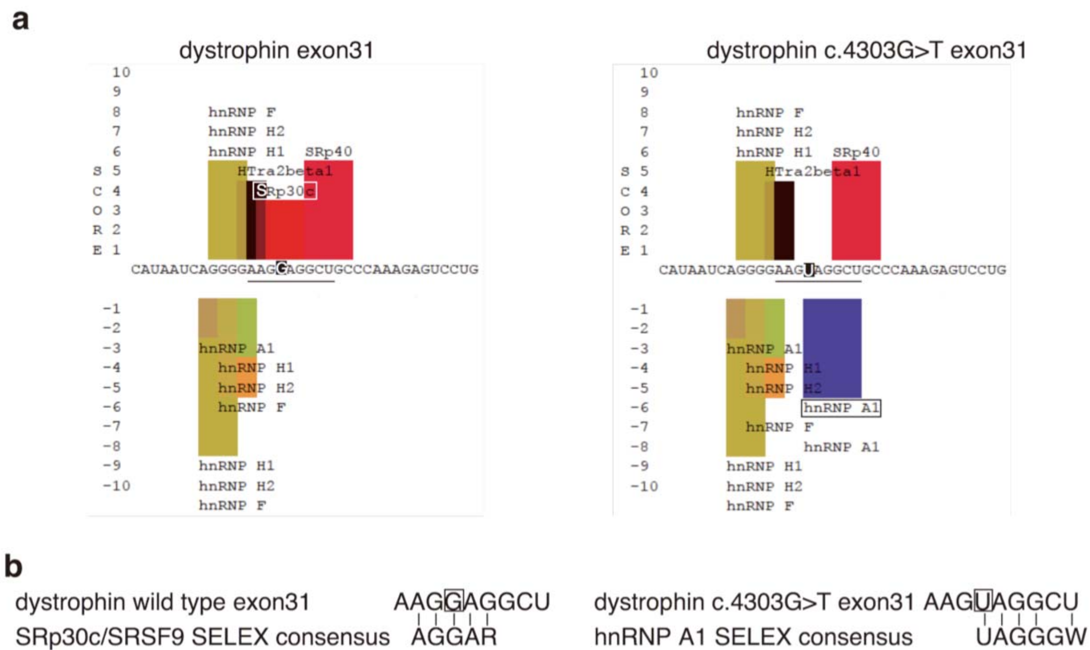


Supplementary Figure S1. TG003 specifically induces skipping of the mutated exon31 and has almost no effect on splicing of other *dystrophin* introns in the patient's muscle cells.

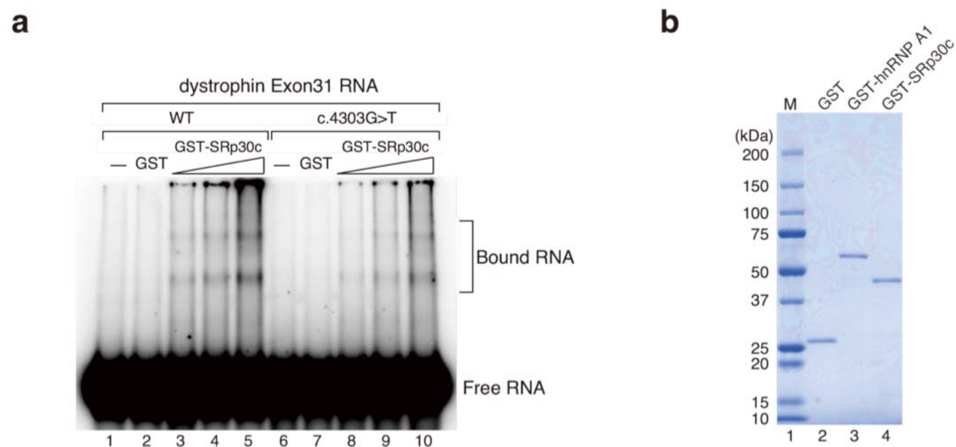
RT-PCR with RNA recovered from primary cultured muscle cells untreated or treated with 30μM of TG003. The numbers of exons amplified by specific primer combinations are indicated above the lanes. The lane marked M contains φX174-HaeIII digest DNA size markers. The lengths of both DNA size markers and PCR products are shown in nucleotides.



Supplementary Figure S2. Prediction of the binding sequences in exon31 for RNA-binding proteins that regulate splicing.

(a) Prediction of the RNA-binding protein candidates that can bind to the wild-type or c.4303G>T exon31 of the *dystrophin* gene by SpliceAid. A positive score was assigned to the sequences that facilitate the defining of exons, such as ESE motifs. With the same criteria, a negative score was assigned to the target sequences that facilitate intron definition, namely ESS motifs. The nucleotide mutated in c.4303G>T is highlighted in both panels. Two proteins, SRp30c/SRSF9 and hnRNP A1, whose scores are drastically changed by this mutation are indicated by open squares. The surrounding sequences that show high homology to the SRp30c/SRSF9 (left panel) or hnRNP A1 (right panel) SELEX consensus sequence are underlined.

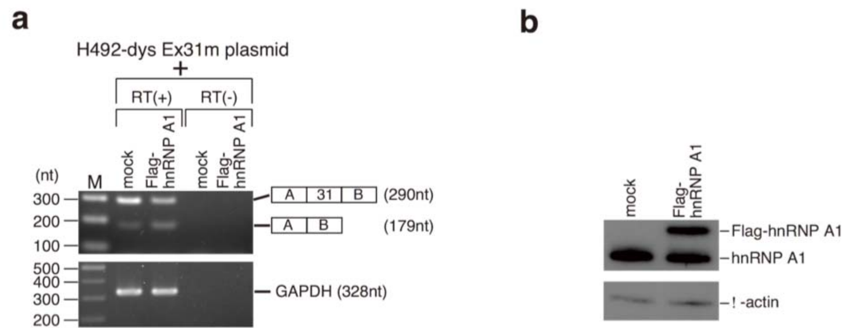
(b) The alignment of the exon31 sequence with the SRp30c/SRSF9 or hnRNP A1 SELEX consensus sequence. The homology between the wild-type exon31 RNA and SRp30c/SRSF9 SELEX consensus sequence is shown in the left panel. R indicates a purine residue. The RNA sequence homology between the mutated exon31 and hnRNP A1 SELEX consensus is indicated in the right panel. W indicates A or U. Vertical bars in both panels designate identical residues. The nucleotide mutated in c.4303G>T is highlighted by an open square in both panels.



Supplementary Figure S3. Point mutation in exon31 of the *dystrophin* gene reduces binding of SRp30c/SRSF9 *in vitro*.

(a) Gel mobility shift assays with GST-SRp30c/SRSF9 and dystrophin exon31 RNA. ^{32}P-labeled dystrophin exon31 RNAs (wild type or mutant) was incubated with either GST alone (shown as GST: lanes 2 and 7, 400ng) or GST-tagged SRp30c (designated as GST-SRp30c: lanes 3-5 and 8-10; 100, 200, and 400ng, respectively), and the resultant complexes were subjected to 8% non-denaturing polyacrylamide gel electrophoresis. Lanes 1 and 6 show where the RNA itself migrates on the gel (marked as Free RNA on the right). Complexes of SRp30c and RNA are also indicated as Bound RNA. All incubations were carried out at 20ºC for 30 min.

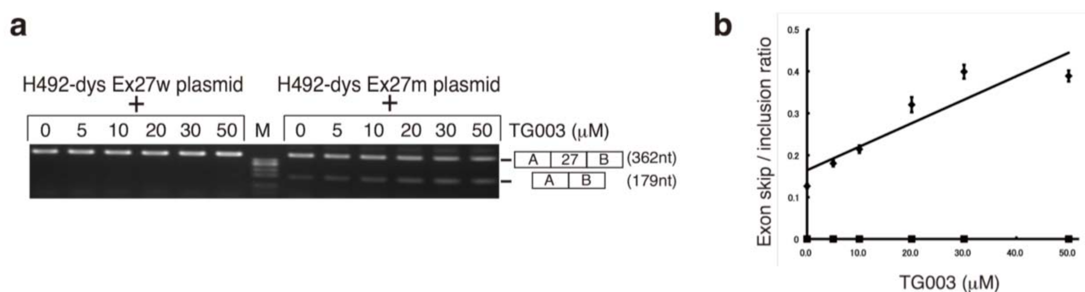
(b) Preparation of recombinant GST-fused proteins in *E.coli*. GST, GST-hnRNP A1 and GST-SRp30c proteins were expressed in *E.coli* cells and purified by using both glutathione and Ni- columns. Five hundred nanograms of each purified protein was analyzed by 5-20% gradient SDS-PAGE. The size of molecular weight markers in kDa is shown at the left.



Supplementary Figure S4. hnRNP A1 overexpression in HeLa cells

(a) Effect of overexpression of hnRNP A1 on the skipping of the mutant exon31. HeLa cells were cultured and transfected with the mutant reporter plasmid in combination with the plasmid that expresses either Flag peptide (mock) or Flag-tagged hnRNP A1 (Flag-hnRNP A1). RNAs were recovered and analyzed by RT-PCR reactions with (RT+) and without (RT-) reverse transcriptase (RT) as shown in Figure 2c. The lane marked M contains 2-Log DNA ladder size markers. The nucleotide lengths of DNA size markers and PCR products are shown at the left and right, respectively.

(b) Western blotting of both endogenous and exogenous hnRNP A1 protein expression in Flag-hnRNP A1 plasmid transfected HeLa cells. Proteins were detected with antibodies recognizing hnRNP A1 (4B10). The antibody against β-actin indicated that equivalent numbers of the cells were used for this assay.



Supplementary Figure S5. TG003 also promotes the skipping of a mutated exon27 in HeLa cells in a dose-dependent manner.

(a) RT-PCR of the RNA recovered from HeLa cells transfected the wild-type (H492-dys Ex27w) or mutant (H492-dys Ex27m) plasmid and cultured in the presence of several concentrations of TG003. The nucleotide lengths and schematic representation of PCR products are indicated at the right of the panel. The lane marked as M contains DNA size markers (ϕX174-HaeIII digest).

(b) Quantitation of the ratio of exon skipping to inclusion for the RNA products expressed from the wild type (closed squares) and mutant (closed circles) reporter plasmids in the presence of TG003. Averages and standard deviations from three independent experiments are shown.

Primers used for RT-PCR to detect mRNAs for dystrophin and GAPDH (Figures 1, 2 and Supplementary Figures S4, S5)

name	hybridized region	sequence
c27f	exon27	CCTGTAGCACAAGAGGCCTTA
reverse 2F	exon32	TCCACACTCTTTGTTTCCAATG
GAPDH FW	exon4	CCATCACCATCTTCCAGGAGCGAG
GAPDH RV	exon6	GTGATGGCATGGACTGTGGTCATG

Primers used for quantitative RT-PCR to detect mRNAs from H492-dys 31m plasmid (Figure 3)

For exon31 included mRNAs

name	hybridized region	sequence
exonA-exon31-F	exonA	ATCTTTCGGAAGGAAGGCAACT
exonA-exon31-R	exon31	TCCCCTGATTATGTTTCTTCATTTC
exonA-exon31 probe	exonA-exon31 junction	AAGTCAATAAAATCCAATCTG

For exon31 skipped mRNAs

name	hybridized region	sequence
exonA-exonB-F	exonA	TCTGAGGCTTGTTTGATGCTATCT
exonA-exonB-R	exonB	ATCTTTCGGAAGGAAGGCAACT
exonA-exonB probe	exonA-exonB junction	CATTAACACCCTATTGACTT

Primers used for quantitative RT-PCR to detect endogenous dystrophin mRNAs from the patient cells (Figure 4)

For exon31 included mRNAs

name	hybridized region	sequence
exon30-31-F	exon30	GTGGACGCAGCTCAAATGC
exon30-31-R	exon31	TCAATCTGAGACAGGACTCTTTGG
exon30-31 probe	exon30-exon31 junction	AGCCCAGAAAATC

For exon31 included mRNAs

name	hybridized region	sequence
exon30-32-F	exon30	AAGGTGGACGCAGCTCAAA
exon30-32-R	exon32	CTGGAATAATCGAAACTTCATGGA
exon30-32 probe	exon30-exon32 junction	AAGCCCAGAAAAAA

Primers used to investigate splicing of all dystrophin introns in the patient's myotubes (Supplementary Figure S1)

name	exons amplified	location	sequence
1C	1-8	1-22	ATGCTTTGGTGGGAAGAAGTAG
c8r	1-8	831-809	CTGTTGAGAATAGTGCATTTGAT
c7f	7-11	556-579	GTGGTTTGCCAGCAGTCAGCCACA
1D	7-11	1230-1208	TCCTGTTCCAATCAGCTTACTTC
1E	10-14	1084-1104	TTGCAAGCACAAGGAGAGATT
c14r	10-14	1689-1669	ACGTTGCCATTTGAGAAGGAT
c13f	13-18	1579-1599	GCTGCTTTGGAAGAACAACTT
1F	13-18	2218-2197	CTTCTGAGCGAGTAATCCAGCT
2C	17-21	2134-2156	AGGCAGATTACTGTGGATTCTGA
c21r	17-21	2803-2783	TTGTCTGTAGCTCTTTCTCTC
c21f	21-25	2650-2669	CAACCTCAAATTGAACGATT
2D	21-25	3336-3316	CCCACCTTCATTGACACTGTT
2E	24-28	3112-3134	GAGCATTGTCAAAAGCTAGAGGA
c28r	24-28	3816-3793	CAATAACTCATGCCAACATGCCCA
c27f	27-32	3688-3708	CCTGTAGCACAAGAGGCCTTA
2F	27-32	4481-4460	TCCACACTCTTTGTTTCCAATG
3C	31-35	4309-4328	GCCCAAAGAGTCCTGTCTCA
c35r	31-35	4881-4862	GTGCACCTTCTGTTTCTCAA
c34f	34-38	4753-4772	GAATGGCTGGCAGCTACAGA
3D	34-38	5360-5338	TTAAACTGCTCCAATTCCTTCAA
3E	36-41	5050-5069	TTTGACCAGAATGTGGACCA
c41r	36-41	5826-5806	TGCGGCCCCATCCTCAGACAA
c40f	40-45	5704-5725	AGCCTACCTGAGCCCAGAGATG
3F	40-45	6502-6483	CTTCCCCAGTTGCATTCAAT
4C	44-48	6367-6393	GCTGAACAGTTTCTCAGAAAGACACAA
c48r	44-48	7053-7033	CAACTGATTCCTAATAGGAGA
c48f	48-52	6937-6957	CAAGGAGAAATTGAAGCTCAA
4D	48-52	7658-7636	CGATCCGTAATGATTGTTCTAGC
4E	51-59	7435-7455	TGGACAGAACTTACCGACTGG
c56r	51-59	8326-8307	GTAACAGGACTGCATCATCG
c55f	55-59	8040-8059	AGAGGCTGCTTTGGAAGAAA
4F	55-59	8746-8725	CCCACTCAGTATTGACCTCCTC
5C	58-68	8619-8638	GACAGAGCAGCCTTTGGAAG
c66r	58-68	9589-9568	GGACACGGATCCTCCCTGTTCG
c64f	64-68	9334-9353	CTCCGAAGACTGCAGAAGGC
5D	64-68	9916-9898	TTTCTGCAGCAGCCACTCT
5E	67-72	9775-9792	ATTGAGCCAAGTGTCCGG
c72r	67-72	10297-10277	TATCATCGTGTGAAAGCTGAG
c70f	70-79	10094-10113	CAGGAGAAGATGTTCGAGAC
5F	70-79	11084-11064	ATCATCTGCCATGTGGAAAAG

Supplementary Table S1. The primers used for RT-PCR and quantitative RT-PCR experiments in Figur es 1-4, and Supplementary Figures S1, S4 and S5.

Name	Nature of compound	Final concentration
TG003	Clk1 inhibitor	50μM
SRPIN340	SRPK inhibitor	50μM
Kinetin	a kind of cytokinin	100μM
Kinetin riboside (KR)	a kind of cytokinin	4μM
3-Nitropropionic acid (3-NP)	succinate dehydrogenase inhibitor	200μM
Prednisolone (PDN)	Synthetic adrenal steroid hormone	20μM
Epigallocatechin gallate	A kind of flavonoid	50μM
Trehalose	Disaccharide	100μM

Supplementary Table S2. The chemical compounds tested for promoting the skipping of the mutant exon31 with the H492-based reporter system in HeLa cells.