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online data

SUPPLEMENTARY ONLINE DATA
Contribution of DEAH-box protein DHX16 in human pre-mRNA splicing

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RESULTS

Mutant DHX16 extracts inhibit *in vitro* splicing in standard HeLa extracts

To test whether the mutant protein would inhibit splicing *in trans*, we isolated whole-cell extracts from HEK-293T cells transfected with mutant or wild-type DHX16 plasmids. DHX16 in extracts from plasmid-transfected cells and in standard nuclear splicing extracts were assayed by Western blotting (Supplementary Figure S1A). The transfected extracts were individually mixed with the HeLa nuclear extracts in a 1:2 volume ratio and then subjected to splicing or complex formation assays. RNA was extracted and analysed for the occurrence of splicing (Supplementary Figure S1B) or the reaction mixture was directly applied to non-denaturing gels to assess the occurrence of splicing complexes (Supplementary Figure S1C). Splicing was partially inhibited in the presence of the DHX16-G724N, -K428A or -S552L extract (Supplementary Figure 1B) and spliceosomal complex B was accumulated in reactions containing DHX16-G424N or -S552L (Supplementary Figure S1C). This mixing experiment indicated that these mutant DHX16 proteins, were able to inhibit splicing *in trans*. The accumulation of unspliced minigene transcripts *in vivo* (Figure 5 in the main paper) and the lack of splicing in extracts from DHX16-G724N probably resulted from a direct inhibition of splicing by the mutant protein.

Table S1 Oligonucleotides used in the present study

ORF, open reading frame.

Oligonucleotide	Sequence (5′→3′)
ORF-5′-EcoRI	GAATTCCATGGCGACGCCGGCGGGTCTG
ORF-3′-XhoI	CTCGAGCCCTAGCTCTTCTCGTGTTTT
K428A-sense	CGAGACAGGCTCAGGGGCGACCACCCAGATCCCG
K428A-antisense	CGGGATCTGGGTGGTCGCCCCTGAGCCTGTCTCG
D520A-sense	GCGTGGTGATGGTGGCTGAGGCACACGAAAGG
D520A-antisense	CCTTTCGTGTGCCTCAGCCACCATCACCACGC
G724N-sense	GCCAATCAGCGAGCTAACAGGGCAGGTCGGG
G724N-antisense	CCCGACCTGCCCTGTTAGCTCGCTGATTGGC
pL53In-forward	TCGATCCGCTTCCTGCCCCT
pL53In-reverse	CCGGGCCACCTCCAGTGCC

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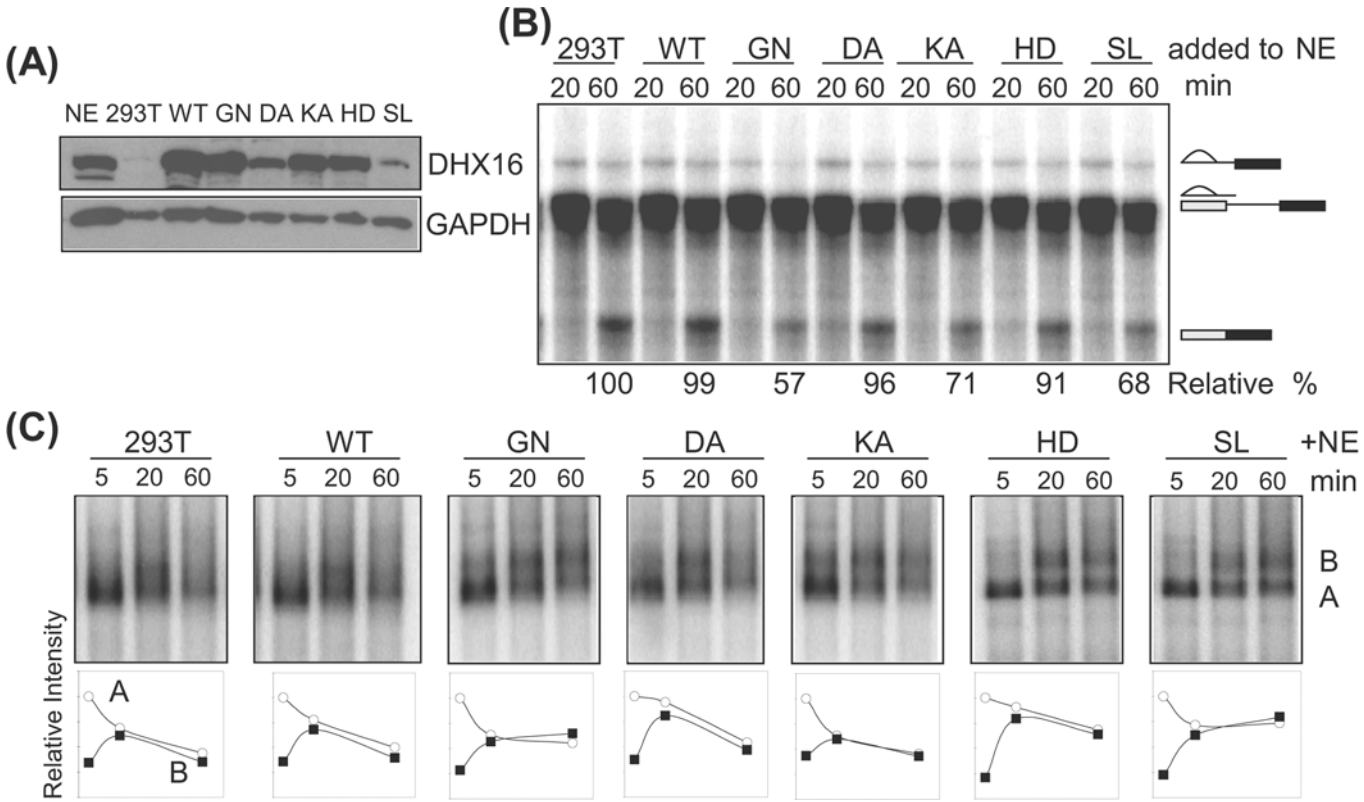

Figure 1 Mutant extracts inhibit splicing and arrest spliceosomal complex B

Whole-cell extracts were prepared from HEK-293T cells transfected with plasmids expressing wild-type or mutant DHX16 proteins. Extracts were added individually to HeLa nuclear splicing extract and the splicing reactions were carried out. Reaction mixtures were withdrawn at the indicated time and analysed. NE, HeLa nuclear splicing extract; 293T, whole-cell extract from untransfected HEK-293T cells. WT, GN, DA, KA, HD and SL are extracts from HEK-293T cells transfected with FLAG-tagged wild-type or a mutant DHX16 expression plasmid (see Figure 5A in the main paper for full details of the mutants). (**A**) Western blot analysis of DHX16 in extracts used 5 μl of whole-cell extracts (10 μl for NE). (**B**) Splicing reactions were carried out in NE (1/3 of the reaction volume) supplemented with the extracts being tested (1/6 of the reaction volume). The spliced RNA intensity from a 60 min reaction was quantified using a PhosphorImager and normalized to the total ^{32}P-labelled RNA. The relative percentage is indicated at the bottom of the gel and is compared with the control. RNA molecules are indicated to the right of the blot and are described for Figure 2(B) in the main paper. (**C**) Splicing complex formation. Splicing reactions as described in (**B**) were performed, stopped by adding heparin to 0.65 μg/μl at the time indicated and analysed on non-denaturing agarose gels. The intensities of complex A and B were quantified using a PhosphorImager, normalized to that of complex A at the 5 min time point of each experiment, and plotted as a relative intensity. Complex A (○); complex B (■).

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