

*Communication*

# **The Exon Junction Complex Core Represses Cancer-specific Mature mRNA Re-splicing: A Potential Key Role in Terminating Splicing**

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### **Fig. S1**

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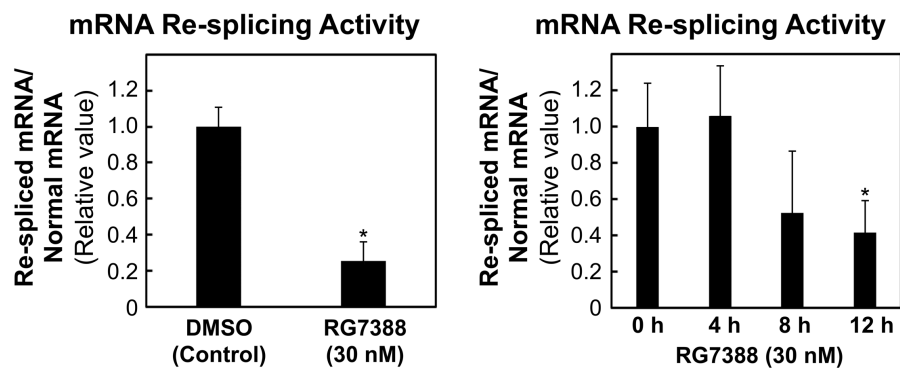
### **Fig. S4**

### **Fig. S5**

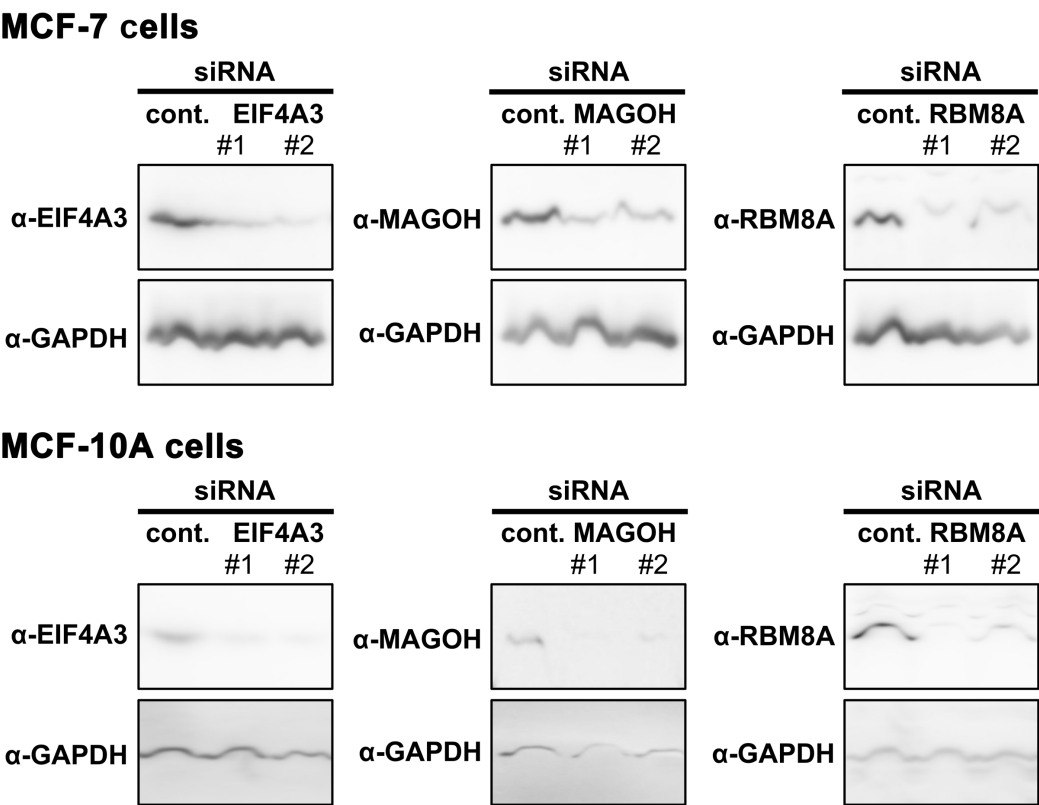
**Table S3** List of the synthetic oligonucleotides used in the experiments (see also Table S2).

siRNAs (sense sequences) for cellular knockdown (T: deoxyribonucleotide)		
EIF4A3 siRNA #1	ID: s18876 (Thermo Fisher Scientific)	Knockdown (KD) of EIF4A3 (shared in Table S2)
EIF4A3 siRNA #2	ID: s18877 (Thermo Fisher Scientific)	
RBM8A (Y14) siRNA #1	ID: s532199 (Thermo Fisher Scientific)	KD of RBM8A
RBM8A (Y14) siRNA #2	ID: s532200 (Thermo Fisher Scientific)	
MAGOH siRNA #1	ID: s226590 (Thermo Fisher Scientific)	KD of MAGOH
MAGOH siRNA #2	ID: s8466 (Thermo Fisher Scientific)	
CASC3 (MNL51) siRNA #1	ID: s22392 (Thermo Fisher Scientific)	KD of CASC3
CASC3 (MNL51) siRNA #2	ID: s22393 (Thermo Fisher Scientific)	
UPF1 siRNA #1	ID: s11927 (Thermo Fisher Scientific)	KD of UPF1
UPF1 siRNA #2	ID: s11928 (Thermo Fisher Scientific)	
NXF1 siRNA #1	ID: s20533 (Thermo Fisher Scientific)	KD of UPF1
NXF1 siRNA #2	ID: s20532 (Thermo Fisher Scientific)	
ACINUS siRNA	5'-gaggccuucuggauugacaTT -3' (Integrated DNA Technologies)	KD of ACINUS
PININ siRNA	5'-ggagcaagggcgauuacuaTT-3' (Integrated DNA Technologies)	KD of PININ
RNPS1 siRNA	5'-caaaggcuauugcguaacuaTT-3' (Integrated DNA Technologies)	KD of RNPS1
SAP18 siRNA	5'-gaacugacaagcuuaguaaTT-3' (Integrated DNA Technologies)	KD of SAP18
Primer DNAs for plasmid construction (F: forward primer, R: reverse primer)		
RBM8A coding-F	5'-ACAAGTGGCCTCTGAGGCCCCACCATGGCGGACGTGCTAGATCT-3'	Construction for final pSB-RBM8A
RBM8A coding-R	5'-TGACGGGCCTGACAGGCCTCAGCGACGTCTCCGGTCTG-3'	
Primer DNAs for mRNA products assay (F: forward primer, R: reverse primer)		
GAPDH-F	5'-GCACCGTCAAGGCTGAGAAC-3'	PCR for detection of GAPDH cDNA
GAPDH-R	5'-TGGTGAAGACGCCAGTCCA-3'	
EIF4A3-F	5'-GATCTTGGCTCCCACAAGAG-3'	PCR for detection of EIF4A3 cDNA
EIF4A3-R	5'-TAGTCACCGAGAGCAAGCAG-3'	
TSG101-F	5'-GAAGTTATCATTCCCACAGCTC-3'	PCR for detection of spliced TSG101
TSG101-R	5'-GACGTACATGCTTCAGGAAGAC-3'	
TSG101Δ154-1054-F	5'-TACAAATACAGAGACCTAACTCTCCC-3'	PCR for detection of re-spliced TSG101
TSG101Δ154-1054-R	5'-GACGTACATGCTTCAGGAAGAC-3'	
RBM8A-F	5'-ATTCATCTCAACCTCGACAGG-3'	PCR for detection of RBM8A cDNA
RBM8A-R	5'-CAAATCCTGGCCATTGAGTC-3'	
MAGOH-F	5'-CAAAGAGGATGATGCATTGTG-3'	PCR for detection of MAGOH cDNA
MAGOH-R	5'-TGTGTTTCATCTCCAATGACGA-3'	
CASC3-F	5'-ACCACCGCCTCATCTGTATC-3'	PCR for detection of CASC3 cDNA
CASC3-R	5'-TGGGCGGGTTATAGTAGGT-3'	
UPF1-F	5'-GCAACGGACGTGGAATACT-3'	PCR for detection of UPF1 cDNA
UPF1-R	5'-CTTGTGCAGGGTCACCTCTT-3'	
GAS5-F	5'-CTTGCCTGGACCAGCTTAAT-3'	PCR for detection of GAS5 cDNA
GAS5-R	5'-CAAGCCGACTCTCCATAC-3'	
NXF1-F	5'-GATTCTTTGCGAGCCTTACC-3'	PCR for detection of NXF1 cDNA
NXF1-R	5'-AGGAGGAGATGACAGACGACAACC-3'	
ACINUS-F	5'-GACGATCATCTAGGGTCAGACAG-3'	PCR for detection of ACINUS cDNA
ACINUS-R	5'-GGAAGGTGTTTCTTGATCCTCTT-3'	

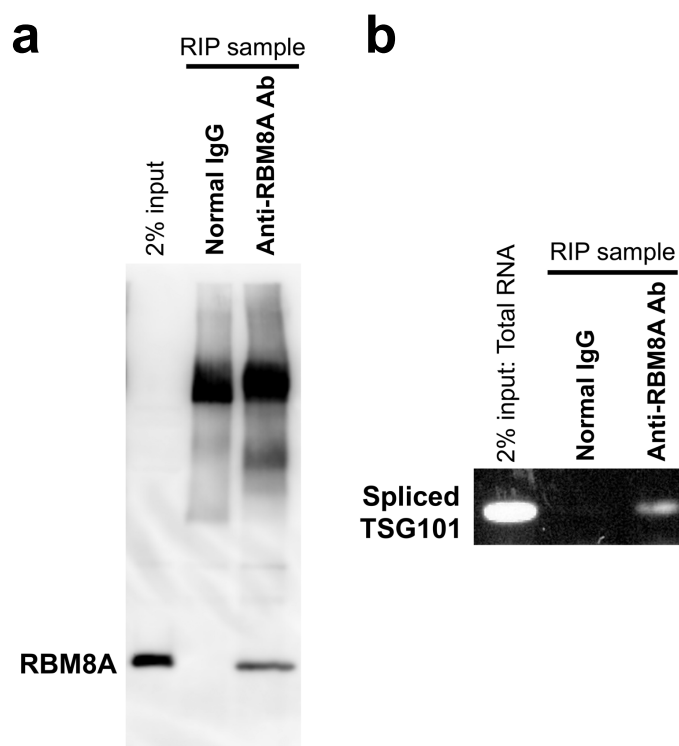
Left, middle, and right columns: names, sequences, and purpose of the oligonucleotides, respectively.



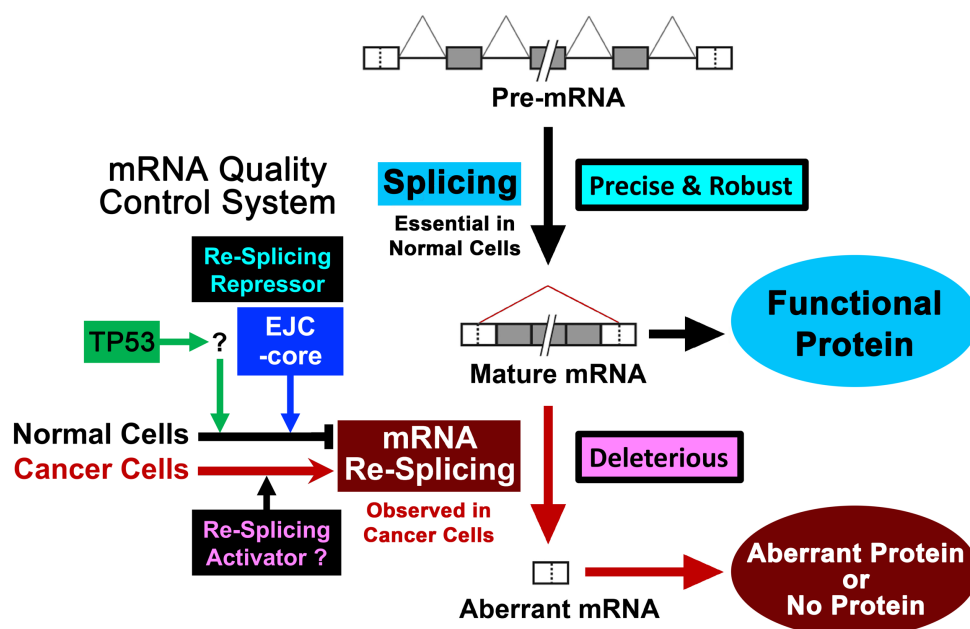
**Figure S1.** Activation of TP53 by the MDM2 inhibitor RG7388 induces the repression of TSG101 mRNA re-splicing activity: To evaluate the re-splicing activity with TSG101 mRNA, the extracted total RNAs from MCF-7 cells were analyzed by RT-qPCR using specific primer sets (see Figure 1a). The RG7388 concentrations and culture time after RG7388 addition were indicated. The histograms represent the means  $\pm$  standard deviations of three replicates (\* $p < 0.05$ ).



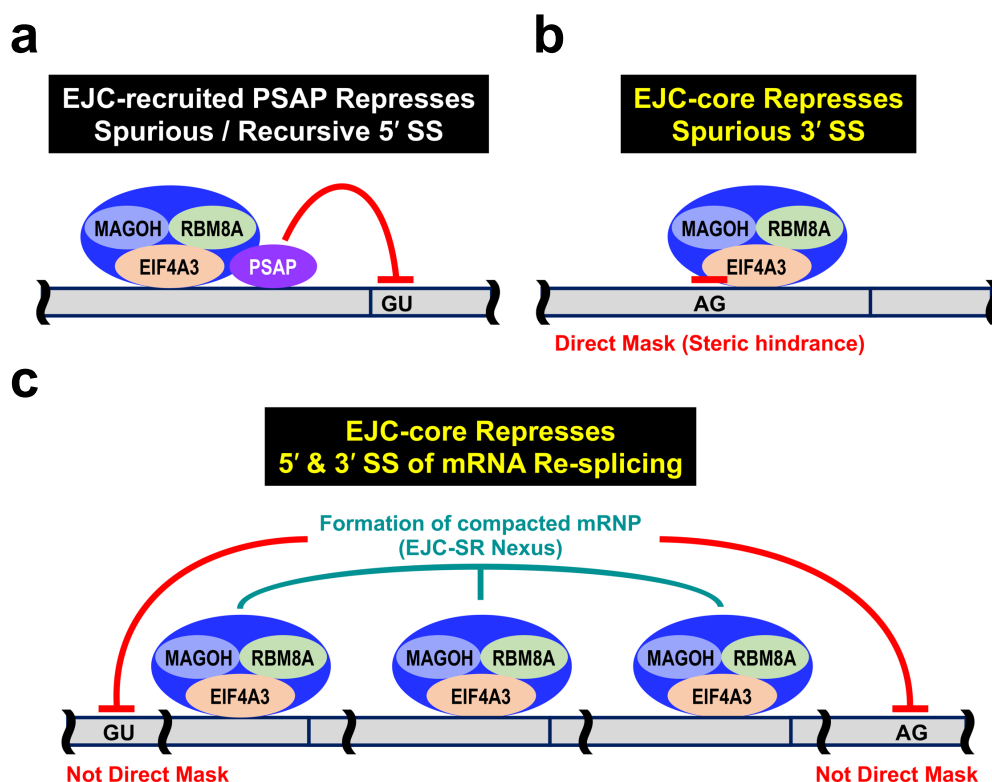
**Figure S2.** The endogenous EJC-core proteins are effectively depleted by the siRNA-mediated knock-down: MCF-7 and MCF-10A cells were transfected with siRNA targeting EIF4A3, RBM8A, MAGOH or control siRNA, and whole cell extracts were analyzed by immunoblotting with the indicated specific antibodies.



**Figure S3.** The EJC core factor RBM8A binds TSG101 mRNAs in MCF-7 cells: (a) RBM8A-bound mRNAs were separated from cell lysates of MCF-7 cells by RNA immunoprecipitation (RIP). Normal rabbit IgG was used as a negative control of the immunoprecipitation. Two independent RIP assays were performed and a representative immunoblot is shown here. (b) The RIP samples were analyzed by RT-PCR using a primer set targeting spliced TSG101 mRNA. The PCR products were visualized by agarose gel electrophoresis.



**Figure S4.** Model of the regulation system of cancer-specific mRNA re-splicing: TP53 represses mRNA re-splicing [10,15; see reference list in the main text], however, the downstream repressor has not been identified. Our discovery of EJC-core as a repressor of mRNA re-splicing implies a mechanism for terminating pre-mRNA splicing in normal cells, which is essential to maintain quality of mRNA.



**Figure S5.** Similarity and distinction with other findings of the EJC-induced splicing repression: Recently, it has been reported that EJC deposition on mRNA represses nearby spurious splice sites [22; see reference list in the main text] and downstream recursive 5' splice site [23]. (a) EJC-recruited PSAP complex (PNN, SAP18 and RNPS1) represses spurious 5' splice site (SS) [22] and downstream recursive 5' SS (but the requirement of PSAP is dependent on the substrate of recursive splicing) [23]. (b) On the other hand, nearby spurious 3' SS is repressed by direct binding of the EJC-core, which causes steric hindrance [22]. (c) Here we showed that EJC-core represses exonic 5' SS and 3' SS used in mRNA re-splicing. Previous CLIP-Seq data suggest that the repression of mRNA re-splicing is not due to the direct binding of EJC-core on the 5' SS and 3' SS [24]. We propose that EJC-core deposition triggers the formation of compacted mRNP leading to the prevention of further re-splicing on mRNA (see Figure 6).