Legends for Supplementary Materials

Fig. S1. Structure of *U2AF1* gene and AS-derived variant proteins. (A) A schematic diagram of *U2AF1* genomic locus with exons depicted in vertical boxes. Alternative splicing scheme is also indicated with the two alternative third exons in blue and red. (B) Spliced transcripts and encoded proteins are schematically shown. S34F mutation is common to both variants. Amino acid sequences from the alternative exon are provided and positioned in relation to functional domains of U2AF1 protein.

Fig. S2. RT-PCR validation of AS events. The Venn diagram in Figure 2A show 40 genes (47 AS events) that undergo same AS events in LUAD patients with *U2AF1* S34F mutation and in *U2AF1* S34F mt overexpressing A549 cells. Gene enrichment analyses indicated 7 genes involved in cell cycle are clustered: *MNAT1*, *PARD3*, *CUL5*, *ERBB2IP*, *FANCI*, *SKA2*, *ANAPC10*. Among these, RT-PCR validation results of *PARD3* and *ERBB2IP* were shown in Figure 1C and D. Results for the rest are shown here. (A-E) Alternatively spliced exon and surrounding exons are diagrammed for each of the five genes. Arrows indicate primers used for RT-PCR. On the right, RNA-seq read coverage is shown. Direction of transcription is indicated above, and positions of differentially spliced exon are indicated below for each gene. For *MNAT1* and *CUL5*, although amplicons of distinct size were expected, only reduced levels of expression relative to WT cases were evident (*ACTB* gene used as control).

Fig. S3. **Comparison of effects of KD by siRNA#1 and siRNA#2.** (A) Real-time PCR analysis in A549 cells after 48 hour treatment with siRNAs. Results are average ± standard error of the mean (SEM) of three independent assays. (*) represents *P*-value of < 0.05 compared to control condition. (B) Alternatively spliced exon and surrounding exons are diagrammed for each of the three genes for which AS was specifically induced by KD with siRNA#1. Note that KD of *U2AF1* induced by siRNA#2 is weaker than that by siRNA#1 but induces qualitatively similar changes in AS from all

three genes. U2AF1 siRNA#2 (5'-CGCCGUCGCAAGAAGCAUA-3') also specifically targets human *U2AF1*.

File S1. AS events induced upon KD of *U2AF1* and overexpression of two *U2AF1* splice variants with S34F mutation in A549 cells. First worksheet in the file presents an overall description of the results (see Fig. 1). Circled numbers in the Venn diagram represent AS event sets in the worksheets with matching numbers. At the bottom, distinct types of AS are diagrammed. Positions of alternatively spliced exons are presented according to the scheme shown. For example, in the case of SE (skipped exon), differentially spliced exon position indicates the span of the whole exon. In the case of A3SS (alternative 3' splice site), the position indicates the span of the exon portion found in KD or mutant expression conditions. Inclusion and exclusion refers to the state of exons in KD or in S34F mutant expression conditions compared to respective control conditions.

File S2. AS events in *U2AF1* S34F-associated LUAD cases examined in conjunction with AS events from KD and mutant expression in A549 cells. First worksheet in the file presents an overall description of the results (see Fig. 2). Circled numbers in the Venn diagram represent AS event sets in the worksheets with matching numbers. At the bottom, distinct types of AS are diagrammed. Positions of alternatively spliced exons are presented according to the scheme shown.

File S3. Legends for Supplementary Materials

Table S1. Oligonucleotide primers used for RT-PCR. The primer pairs are designed to produce distinct amplicons from alternatively spliced transcripts.