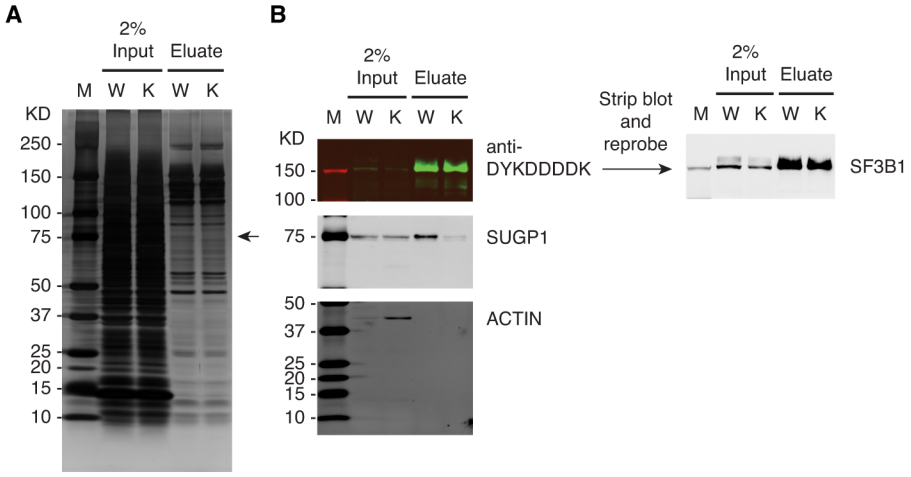

Figure S1. The K700E Mutation Specifically Impairs Association with SUGP1, Related to Figure 2
(A and B) SF3B1-associated proteins were purified from CRISPR/Cas9 engineered K562 cells expressing mono-allelic His6-FLAG-tagged WT (W) or K700E (K) SF3B1 using the large-scale protocol, and then resolved by SDS-PAGE, followed by silver staining (A) or western blotting (B). M, Precision Plus Protein marker (Bio-Rad).

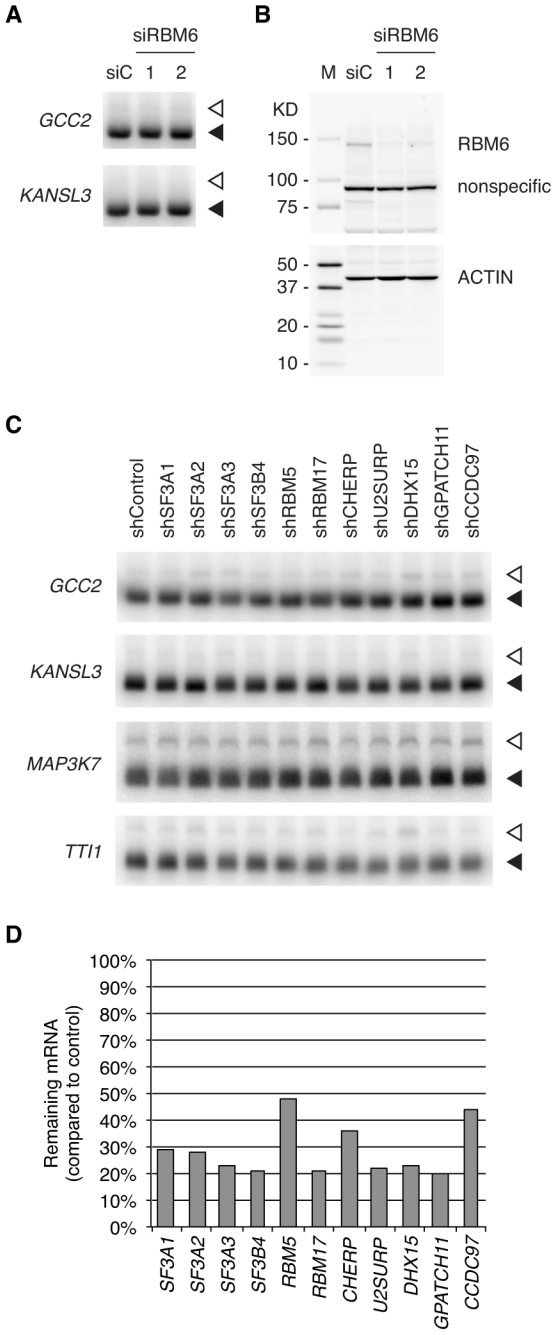

Figure S2. Knockdown of Control Genes Does Not Recapitulate the Splicing Defects of K700E SF3B1, Related to Figure 3

(A and B) HEK293T cells were transfected with a negative control siRNA (siC) or one of two independent siRNAs targeting RBM6, followed by RT-PCR of the cryptic 3′ss (open arrowheads) and canonical 3′ss (solid arrowheads) of *GCC2* and *KANSL3* (A), or western blotting (B). M, Precision Plus Protein marker (Bio-Rad).

(C and D) HEK293T cells were transfected with a non-target control shRNA or one of the shRNAs targeting the indicated genes, followed by RT-PCR of the cryptic 3′ss (open arrowheads) and canonical 3′ss (solid arrowheads) of *GCC2*, *KANSL3*, *MAP3K7*, and *TTI1* (C), or quantification of the remaining mRNA levels of the indicated genes by RT real-time PCR using *RPL13A* as an internal control (D).

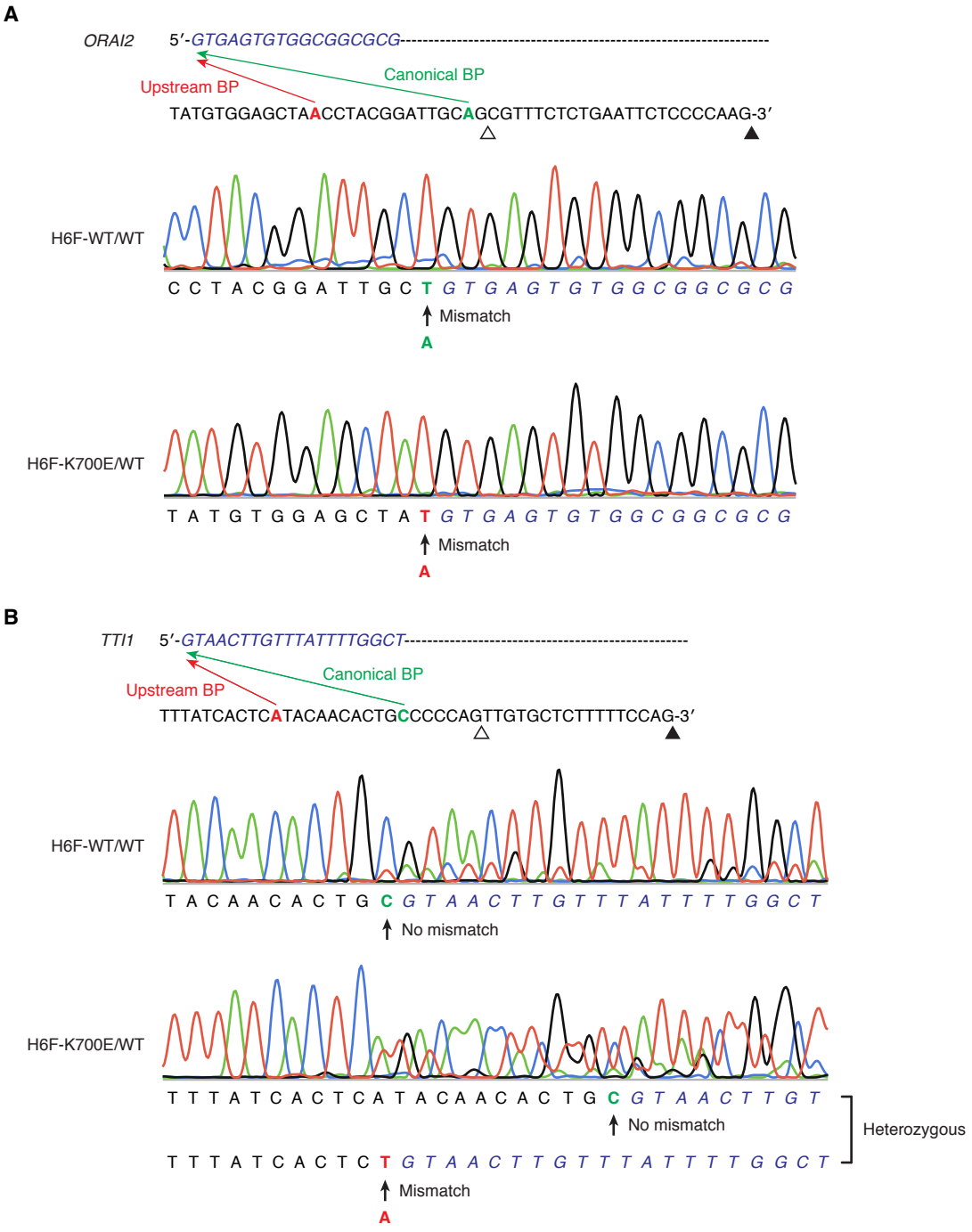

Figure S3. Mapping of Branch Points on Transcripts of *ORAI2* and *TTI1*, Related to Figure 3

(A) Total RNA was extracted from CRISPR/Cas9 engineered K562 cells expressing mono-allelic His6-FLAG-tagged WT (H6F-WT/WT) or K700E (H6F-K700E/WT) SF3B1, and then reverse transcribed using random hexamer primers, followed by nested PCR and sequencing of the intron-lariat intermediates generated from splicing of endogenous transcripts of *ORAI2* (A) and *TTI1* (B). A to T mismatches were observed in the sequencing results, which typically result from nucleotide misincorporation at the BP by the reverse transcriptase reading through the 2′,5′-phosphodiester linkage between the BP and the 5′-end of the intron (Gao et al., 2008).

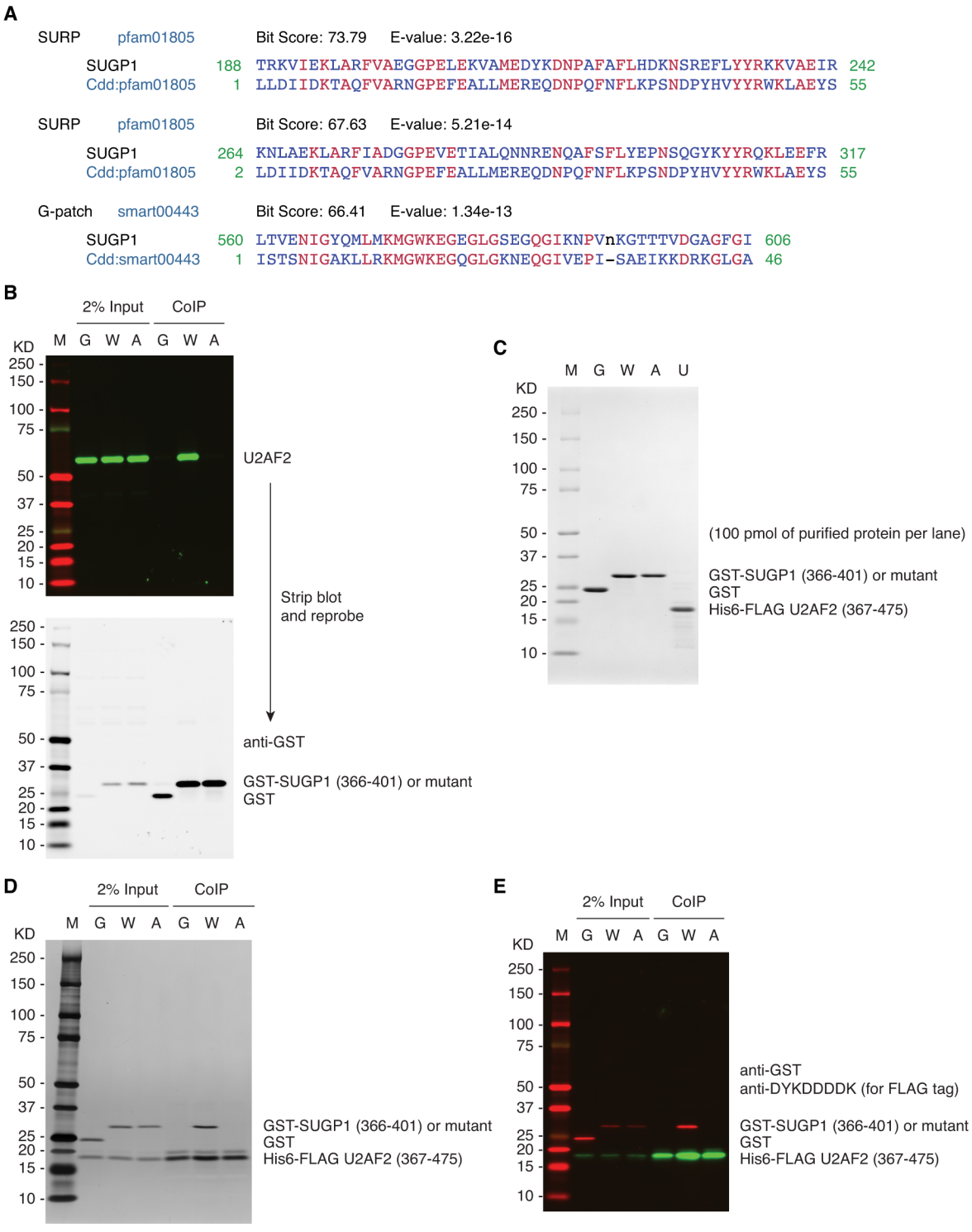

Figure S4. SUGP1 Functional Domains and Interaction with U2AF2, Related to Figure 4

(A) Homology of SUGP1 SURP and G-patch domains to annotated domains in the Conserved Domain Database (Marchler-Bauer et al., 2017).
(B) HEK293T cells were transfected with expression plasmid for GST (G), or GST-tagged WT (W) or W387A (A) mutant SUGP1 (amino acids 366–401). Coimmunoprecipitation (CoIP) was performed with Glutathione Sepharose 4B beads, followed by western blotting. M, Precision Plus Protein marker.
(C) Purified GST (G), GST-tagged WT (W) and W387A (A) mutant SUGP1 (amino acids 366–401), as well as His6-FLAG-tagged U2AF2 (amino acids 367–475) (U) were resolved by SDS-PAGE, followed by staining with QC Colloidal Coomassie Stain. M, Precision Plus Protein marker (Bio-Rad).
(D and E) Purified GST (G), GST-tagged WT (W) or W387A (A) mutant SUGP1 (amino acids 366–401) were CoIPed with His6-FLAG-tagged U2AF2 (amino acids 367–475) (U), followed by silver staining (D) or western blotting (E). M, Precision Plus Protein marker (Bio-Rad).

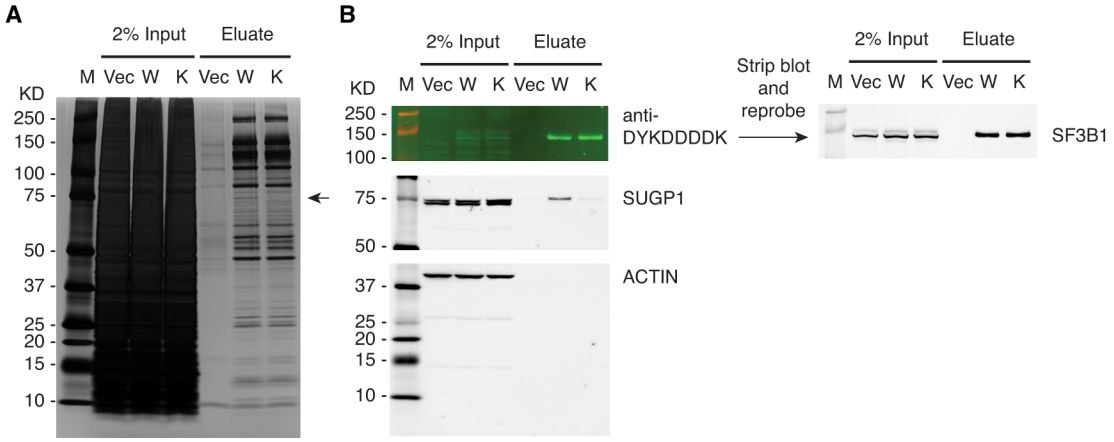

Figure S5. Defective Interaction between Exogenously Expressed K700E SF3B1 and SUGP1, Related to Figure 5
(A and B) HEK293T cells in 10-cm plates were transfected with empty vector plasmid (Vec), or expression plasmid for His6-FLAG-tagged WT (W) or K700E (K) SF3B1, followed by affinity purification using the small-scale protocol. The SF3B1-associated proteins were resolved by SDS-PAGE, followed by silver staining (A) or western blotting (B). Marker, Precision Plus Protein marker (Bio-Rad).

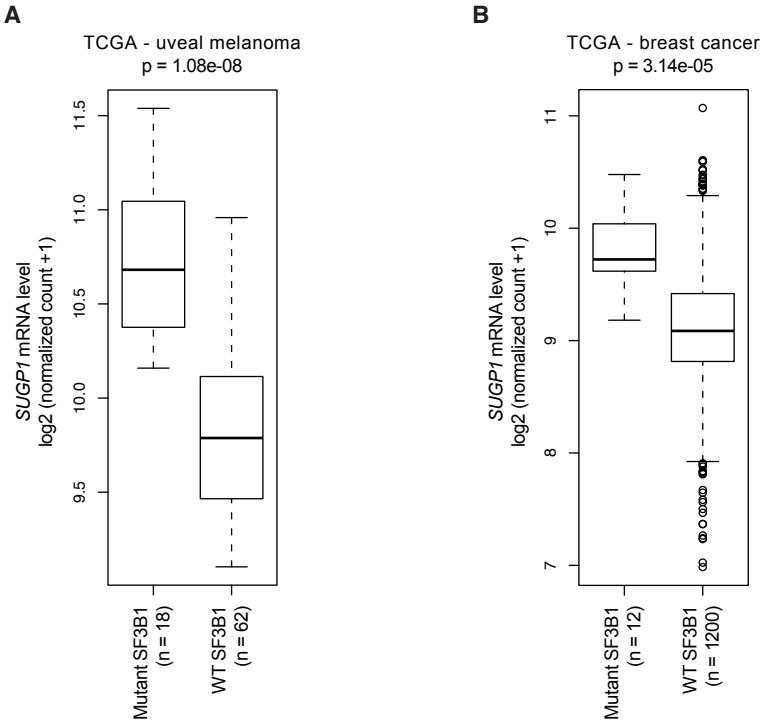

Figure S6. Elevated *SUGP1* mRNA levels in Mutant vs. WT SF3B1 Cancer Samples, Related to Figure 5
(A and B) *SUGP1* mRNA levels in uveal melanoma (A) and breast cancer (B) samples. Normalized *SUGP1* expression data in TCGA samples were downloaded from the UCSC Xena platform (https://xenabrowser.net/).

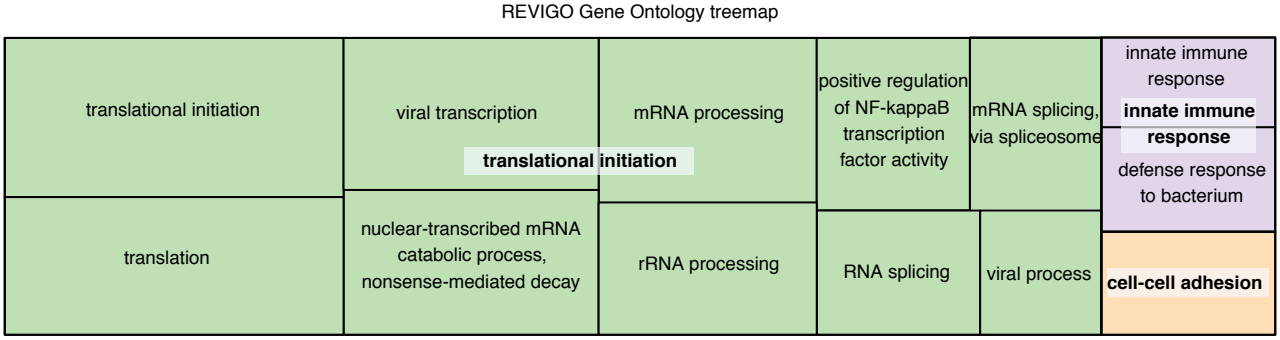

Figure S7. Gene Ontology Analysis of the Functional Pathways Affected by RNA Missplicing, Related to Figure 1
The functional pathways related to the genes associated with the 169 cryptic 3′ss differentially used by mutant vs. WT SF3B1 (see main text) were annotated by a standard gene ontology analysis using DAVID, followed by visualization of the most informative ontology themes using REVIGO.