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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

RESEARCH LETTER

Genome-Scale Analysis Identifies Novel Transcript-Variants in Esophageal Adenocarcinoma

Cancer-associated gene isoforms, arising from aberrant RNA splicing and/or processing, can play a functional role in tumor pathogenesis[1] and are attractive as biomarkers and targets for cancer therapy. To date, the prevalence and significance of such alternative transcript isoforms in esophageal adenocarcinoma (EAC), an increasingly prevalent and lethal malignancy,[2] remain unknown. Here, using an agnostic genome-scale approach, we sought to identify and characterize aberrant cancer-associated transcript-variants in EAC.

Whole transcriptome sequencing (RNAseq) was performed on a discovery sample set of 49 treatment-naive EAC and 40 normal/premalignant fresh-frozen biopsy tissues (Supplementary Table 1 and Supplementary Methods), followed by *de novo* transcriptome analysis to specifically identify novel/unannotated gene transcript-variants primarily induced in EACs but not in normal/premalignant tissues. Following stringent and orthogonal evaluation using transcript-variant specific polymerase chain reaction (PCR) in respective primary EAC tumors, we identified 7 novel candidate EAC-associated transcript-variants (Supplementary Figure 1, Supplementary Table 2). Together, the 7 candidate transcript-variants accounted for 71% of EACs tested, with each of the transcript-variants being induced in 10%–30% of EACs in the RNAseq discovery cohort.

We subsequently prioritized a novel transcript-variant of the collagen X alpha 1 chain precursor (*COL10A1*) gene for further studies, on the basis of the recognized pro-tumorigenic role of COL10A1 pathway network in other tumor contexts.[3–8] Using bidirectional rapid amplification of cDNA ends (RACE) analysis, we first characterized the full-length transcript structure of this novel COL10A1-variant, hereafter referred to as *COL10A1*^{Var1} (deposited

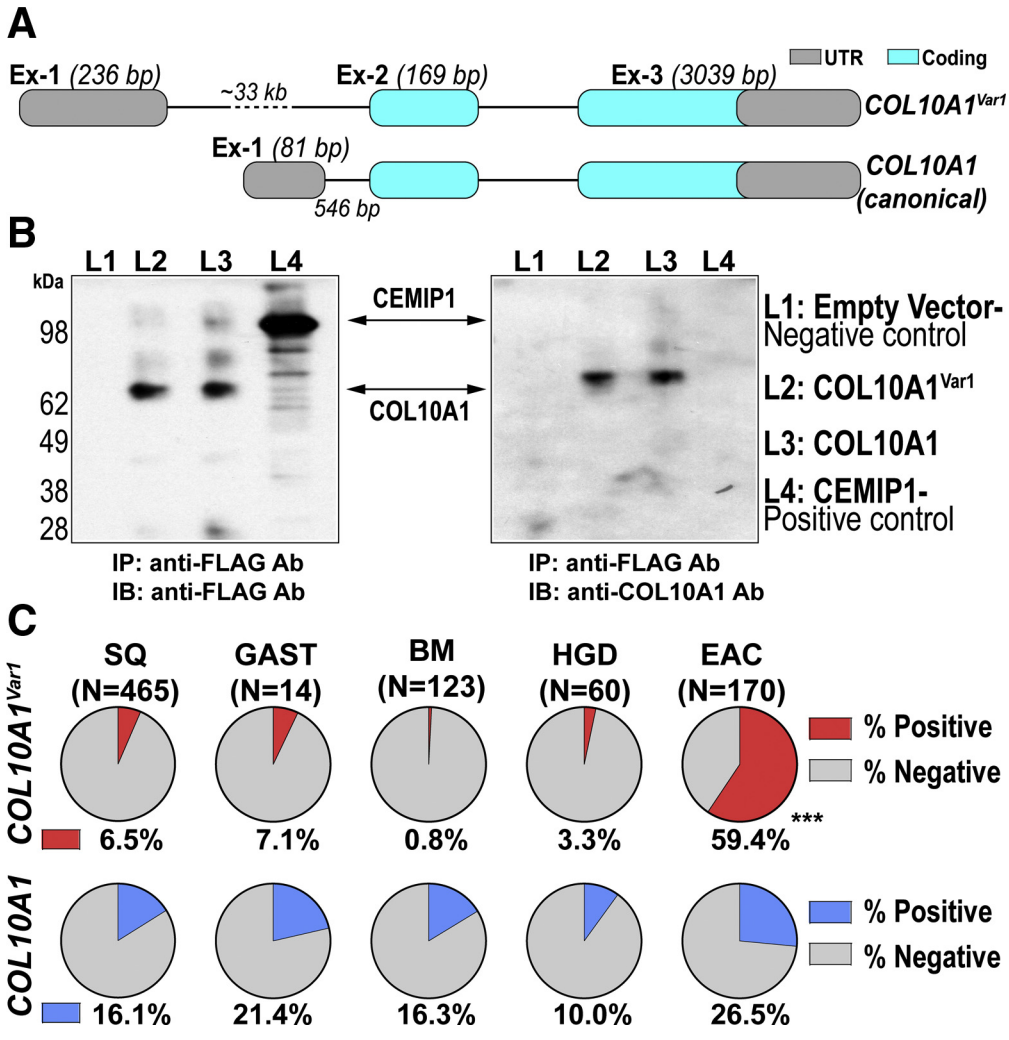

Figure 1. Characterization of *COL10A1*^{Var1}. (*A*) Shown are the 5′ to 3′ exon (Ex)-introns (*thin line*) structures of *COL10A1*^{Var1} and canonical *COL10A1*. UTR, untranslated region. (*B*) Western blot analyses depicting COL10A1^{Var1} and COL10A1 proteins. IB, immunoblotting; IP, immunoprecipitation. CEMIP1 was used as positive control for secreted protein and Empty vector as a negative control. (*C*) Pie charts demonstrating the proportion (%) of samples positive for *COL10A1*^{Var1} transcript (*top, red color*) or canonical *COL10A1* (*bottom, blue color*) in respective SQ, GAST, BM, HGD, and malignant (EAC) tissue biopsies. ***$P < .0001$ indicates significant difference in the proportion *COL10A1*^{Var1} positivity between malignant (EAC) vs any of the respective non-EAC tissue groups, estimated by using a one-tailed Fisher exact test.

in GenBank: MN308081). *COL10A1*^{Var1} is a 3-exon transcript (3444 base pairs [bp]), containing a longer and distinct 5′ exon compared with the canonical (NM_000493.4) transcript (Figure 1*A*, Supplementary Figure 1). In silico analyses (NCBI ORFfinder) predicted *COL10A1*^{Var1} to encode for a ~66 kDa (680 aa) protein, identical in size to the secreted canonical COL10A1 protein, which we confirmed by using orthogonal immunoprecipitation and Western blot analyses upon transfecting HEK293T cells with full-length *COL10A1*^{Var1} transcript (3444 bp), or the coding sequence of canonical *COL10A1* transcript (Figure 1*B*).

Using a robust quantitative real-time PCR (qPCR) assay that specifically detects *COL10A1*^{Var1} but not the canonical transcript, we next evaluated the generality and frequency of *COL10A1*^{Var1} expression in a validation cohort (N = 832) consisting of treatment-naive EAC (N = 170), Barrett's metaplasia (BM) (N = 123), Barrett's with high grade dysplasia (HGD) (N = 60), normal esophageal squamous (SQ) (N = 465), and normal gastric (GAST) (N = 14) biopsy tissues (Supplementary Table 1). Our orthogonal analysis demonstrated *COL10A1*^{Var1} to be robustly induced in the majority (~60%) of EACs (Figure 1*C*, Supplementary Table 3). In striking contrast to EAC, only a minority of BM, HGD, SQ, and GAST samples tested positive for *COL10A1*^{Var1} (Fisher exact test, $P < .0001$; Figure 1*C*, Supplementary Table 3). We also note that *COL10A1*^{Var1} is a more frequently detected isoform in EACs, as compared with the canonical *COL10A1* transcript that was detected in approximately one-fourth of EAC samples with no marked differences between EAC and normal/premalignant tissues (Figure 1*C*, Supplementary Table 3). Taken together, these findings strongly point to *COL10A1*^{Var1} as a recurrently induced transcript-variant in advanced stages of EAC development.

Because fibrillary protein networks (collagen, elastin) and glycoproteins (fibronectin) play a vital role in facilitating migration and invasion of cancer cells,[9] we next evaluated the impact of *COL10A1*^{Var1} knockdown on the migratory potential of EAC cells in a durotaxis[10] assay. We note that the EAC cell lines positive for *COL10A1*^{Var1} also expressed canonical *COL10A1* transcript (Figure 2*A*), and repeated attempts to specifically knockdown *COL10A1*^{Var1} with custom short hairpin RNAs (shRNAs) proved technically unsuccessful. Nonetheless, because both *COL10A1*^{Var1} and canonical *COL10A1* transcripts code for identical protein (Figure 1*B*) and consequently may exhibit similar function, as an alternative approach we used well-characterized *COL10A1* shRNAs that also target *COL10A1*^{Var1} for subsequent studies. OE19 EAC cells (Figure 2*A*), stably expressing control or *COL10A1* shRNAs under the control of doxycycline (Figure 2*B*), were seeded onto one-half of a glass coverslip coated with fibronectin alone (representing soft surface). Migration (durotaxis) of cells from the soft surface to an adjacent fibronectin-coated hydrogel (stiffer, 12 kPa) surface was monitored over time in the presence of doxycycline. Loss of *COL10A1*^{Var1}/*COL10A1* indeed significantly impeded the durotactic ability of EAC cells ($P < .004$) (Figure 2*C*), suggesting *COL10A1* isoforms as potential regulators of mechanosensing ability of EAC cells.

Taken in toto, we identify *COL10A1*^{Var1} as a novel and recurrent EAC-associated transcript-variant with a potential pro-tumorigenic function. On a broader scale, our study represents the first genome-wide analysis identifying novel transcript-variants induced in EAC.

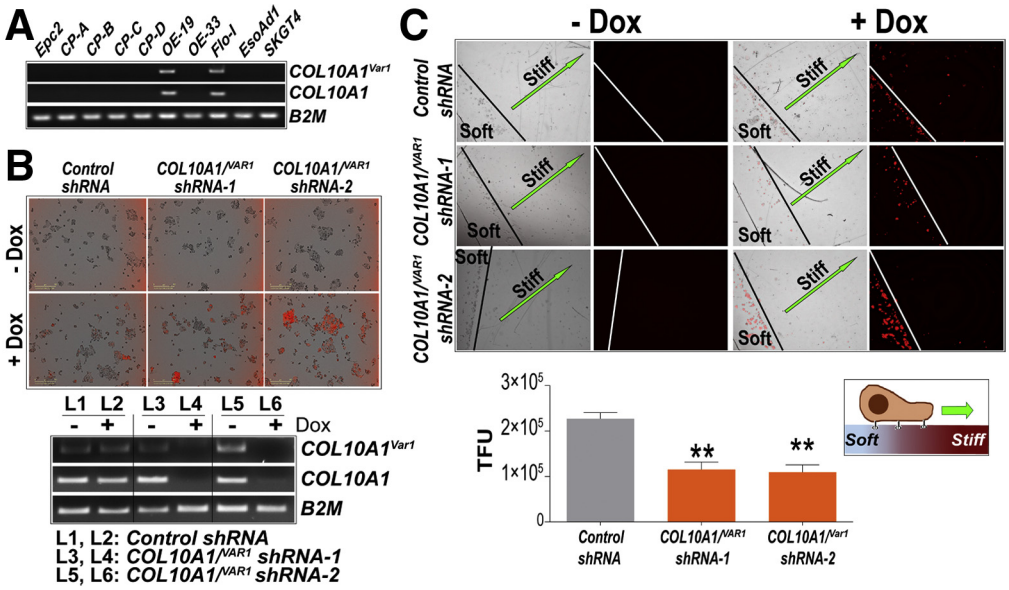

Figure 2. Impact of *COL10A1*^{Var1} on durotaxis of EAC cells. (*A*) PCR-based analysis showing *COL10A1*^{Var1} and canonical *COL10A1* expression in normal esophageal squamous (Epc2), non-dysplastic BE (CP-A), dysplastic BE (CP-B, CP-C, CP-D), and EAC (OE19, OE33, FLO-1, EsoAd1, SKGT4) cell lines. *B2M* was used as the internal RNA control. BE, Barrett's esophagus. (*B*) Representative images (*left*) demonstrating shRNA induction on doxycycline (Dox) treatment in stable OE19 cells, carrying either non-targeting control shRNA or shRNAs targeting both *COL10A1*^{Var1} and canonical *COL10A1* transcripts (depicted as *COL10A1*/^{Var1}). Note the specific induction of TurboRFP, a red fluorescent reporter of shRNA induction, on doxycyline treatment in these cells. PCR analysis (*right*) demonstrating knockdown of *COL10A1*/^{Var1} RNA on doxycycline treatment of the stable OE19 cells. *B2M* was used as an internal RNA control. (*C*) Representative images of durotaxis assay in stable OE19 cells. Quantitative analysis of cell migration (*bar graph*), measured as total fluorescence units (TFU, Y-axis) of TurboRFP-positive cells in the stiffer surface. All data are plotted as mean ± standard error of the mean, obtained from 3 replicate experiments. $^{**}P < .004$ indicates significant differences in *COL10A1*/^{Var1} knockdown vs control shRNA cells, estimated by using a Student *t* test assuming unequal variances.

Further comprehensive studies are warranted to decipher the biologic role of the identified candidates and to evaluate their utility as biomarkers and therapeutic targets in this increasingly prevalent and lethal malignancy.

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Abbreviations used in this letter: **BM, Barrett's metaplasia; bp, base pair; COL10A1, collagen X alpha 1 chain precursor gene; EAC, esophageal adenocarcinoma; GAST, normal gastric; HGD, Barrett's with high grade dysplasia; PCR, polymerase chain reaction; qPCR, quantitative PCR; RACE, rapid amplification of cDNA ends; shRNA, short hairpin RNA; SQ, normal esophageal squamous**

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Durgadevi Ravillah (Formal analysis: Equal; Methodology: Equal),
Lakshmeswari Ravi (Methodology: Supporting),
Rajesh Gupta (Methodology: Supporting; Resources: Supporting),
Marcia I. Canto (Resources: Equal)
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Amitabh Chak (Data curation: Lead; Funding acquisition: Lead; Project administration: Equal; Resources: Lead; Supervision: Equal; Writing – review & editing: Equal),
Vinay Varadan (Data curation: Equal; Formal analysis: Lead; Funding acquisition: Supporting; Investigation: Equal; Methodology: Lead; Supervision: Equal; Writing – review & editing: Lead),
Kishore Guda (Conceptualization: Lead; Formal analysis: Lead; Funding acquisition: Lead; Investigation: Lead; Methodology: Lead; Supervision: Lead; Validation: Lead; Writing – original draft: Lead; Writing – review & editing: Equal)

Conflicts of interest

This author discloses the following: V. Varadan is a consultant/advisory board member for Curis, Inc. The remaining authors disclose no conflicts.

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Supplementary Methods

Patient Samples

We compiled an in-house whole-transcriptome RNA sequencing (RNAseq) dataset previously generated by our group[1,2] for discovery studies and an independent validation cohort (N = 832) consisting of treatment-naive malignant, premalignant, and nonmalignant biopsy tissues (Supplementary Table 1). All samples were accrued with informed consent under an institutional review board approved protocol (UHCMC IRB, #CC301) as previously described.[2]

Identification of Novel Transcript-Variants Using Whole-Transcriptome RNA Sequencing

Briefly, RNAseq reads that passed quality control were aligned to the human reference genome (GRCh37p13) using the STAR aligner v2.5.1. The resulting bam files were sorted for *de novo* transcriptome assembly using Cufflinks in addition to the Gencode transcriptome annotation for GRCh37 version 19 as a guide. The resulting final merged transcriptome assembly was compared with the reference annotation from Gencode using Cuffcompare. Of note, the detection of any transcript, including novel transcripts, in the *de novo* transcriptome assembly was required to be supported by a minimum of 10 paired-reads. Novel transcripts were further examined visually to confirm the presence of supporting reads spanning the novel junctions using the Integrative Genomics Viewer (IGV). Subsequent experimental validations of these junctions were performed by using PCR analysis with custom intron-spanning primer sets.

Quantitative Real-time Polymerase Chain Reaction

One microgram of total RNA was reverse-transcribed by using Superscript III First-Strand Synthesis (Life Technologies, Carlsbad, CA; #18080). Quantitative PCR analysis was performed by using iQ SYBR Green Supermix system (Bio-Rad Laboratories, Hercules, CA; #170-8887) with custom intron-spanning primer set for *COL10A1*^{Var1} (Supplementary Table 2) or commercially available primer set for canonical *COL10A1* transcript (Qiagen, Hilden, Germany). *B2M* was used as an endogenous RNA control as previously described by our group.[2] Each qPCR reaction was carried out in triplicate in a 25 μL volume for 50 cycles using a Bio-Rad CFX96 Real-Time PCR machine. Samples were designated positive for *COL10A1* transcript isoforms using respective melt-curve signals in the qPCR assay. Representative qPCR products were further subjected to direct Sanger sequencing for additional confirmation of transcript isoforms. A negative sample indicates no signal in a 50-cycle qPCR assay.

Rapid Amplification of cDNA Ends

We obtained the full-length sequence of the novel transcript-variant, *COL10A1*^{Var1}, through RACE in OE19 EAC cell line using the SMARTer RACE cDNA kit (Takara Bio, Kusatsu, Shiga, Japan; #634860). The RACE products were purified, cloned into TOPO TA vector (ThermoFisher Scientific, Waltham, MA), and subsequently confirmed by Sanger sequencing.

Cell Culture and Transfection

EAC and premalignant Barrett's esophagus cell lines were cultured as previously described by our group.[1,2] HEK293T cells were transfected with pcDNA3.1 vector containing either FLAG-tagged canonical *COL10A1* ORF (GenScript USA Inc, Piscataway, NJ; #OHU18227D), full-length *COL10A1*-*Var1*, empty vector (negative control), or with *CEMIP* [3] (positive control for secreted protein) using Lipofectamine 2000 (Life Technologies; #11668019).

Immunoprecipitation and Immunoblotting

Cell culture supernatants were transferred to an Amicon Ultra-4 10K filter column (Millipore, Burlington, MA; #UFC801024), concentrated by centrifugation at 4000*g* for 15 minutes, immunoadsorbed overnight at 4°C using anti-FLAG antibody conjugated agarose beads (Sigma-Aldrich, St. Louis, MO; #A2220), and washed with RIPA buffer (150 mmol/L NaCl, 25 mmol/L Tris [pH 7.4], 0.1% sodium dodecyl sulfate, 1% NP-40). The immunoprecipitated proteins were subjected to electrophoresis on 4%–12% polyacrylamide gel (Life Technologies; #0321) and transferred to Hybond–C Extra nitrocellulose membrane (GE Healthcare, Chicago, IL; #10600016). Membranes were blocked with 5% milk in TBST (0.05% Tween-20 in Tris buffered saline) and incubated overnight at 4°C with either horseradish peroxidase–conjugated anti-FLAG antibody (Cell Signaling Technology, Danvers, MA; #2044S) or anti-COL10A1 (Abcam, Cambridge, UK; #ab182563) primary antibody at 1:1000 dilution. For COL10A1, blots were incubated with anti-rabbit horseradish peroxidase secondary antibody (Cell Signaling Technology; #7074) at 1:5000 in 5% milk in TBST. Chemiluminescence was visualized by using ECL-Plus Western Blotting Detection Kit (GE Healthcare; #RPN2232).

Stable OE19 Cell Line Generation With Conditional *COL10A1*^{Var1} Knockdown

Doxycycline-regulated TurboRFP lentiviral vectors, containing nonoverlapping shRNAs targeting different regions of COL10A1/Var1 transcript (Dharmacon, Lafayette, CO; #V3SH11252-227571902, #V3SH11252-228435149) or non-targeting shRNA (Dharmacon; #VSC11655), were produced in HEK293T cells using standard procedures, and viral titers were analyzed by using a 24-*gag* ELISA kit (Takara; #632200). OE19 EAC cells were infected with the viral particles and treated with puromycin (500 ng/mL) for subsequent stable cell line generation. Induction of shRNAs on doxycyline (0.6 μg/mL) treatment was confirmed by TurboRFP signal under fluorescent microscope, and knockdown of *COL10A1*/^{Var1} was confirmed by qPCR with isoform-specific primers. At least 3 independently derived clones per shRNA were used for the study. These lentiviral-based shRNAs were used in the durotaxis assay as described below.

Durotaxis Assay

Durotaxis assay was performed following protocol of Wen et al[4] with some modification. Cells were seeded onto one-half of 18 mm^2 glass coverslip coated with fibronectin alone (representing soft surface), whereas the second-half of the glass coverslip contained a fibronectin-coated polyacrylamide hydrogel, representing the stiffer (12 kPa) surface. Briefly, the coverslip was functionalized by using 3-(trimethoxysilyl) propyl methacrylate (Millipore Sigma; #440159) to facilitate covalent attachment of hydrogel substrates to glass surface. A polymer solution containing acrylamide monomers (Millipore Sigma; #A7802), cross-linker N,N methylene-bis-acrylamide, ammonium persulfate (Millipore Sigma; #A3678), and N,N,N0,N0-tetramethylethylenediamine (TEMED) (Bio-Rad; #1610801) was prepared and allowed to polymerize on one-half of the glass coverslips. The 6.1% acrylamide was used to obtain the 12 kPa of hydrogel stiffness. The gels were sterilized through ultraviolet exposure for 2 × 30 minutes. To allow for cell adhesion and fibrous-protein tethering, substrates were incubated in 1 mmol/L N-sulphosuccinimidyl-6-(40-azido-20-nitrophenylamino) hexanoate (sulpho-SANPAH) (Millipore Sigma; #803332), activated with ultraviolet light exposure for 2 × 5 minutes, followed by 1× phosphate-buffered saline wash for 3 times. The entire glass coverslips were then incubated in fibronectin (ThermoFisher Scientific; #PHE0023) overnight, followed by normalization with cell culture medium for at least 2 hours. The 1×10^4 OE19 EAC cells, expressing $COL10A1/^{Var1}$ shRNAs or control shRNA (see above), were seeded on one-half of the coverslip with the fibronectin-coated glass surface and allowed them to grow overnight. Subsequently, the cells were treated with 10% (Tet-free) fetal bovine serum supplemented culture media with or without doxycycline (0.6 μg/mL). Experiments were performed in triplicates, and the fluorescent signals were captured and measured over time with Keyence BZ-X800 (Osaka, Japan) fluorescence microscope and analyzed with the Keyence image analyzer.

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COL10A1^{Var1} mRNA, complete (3,444 bp)

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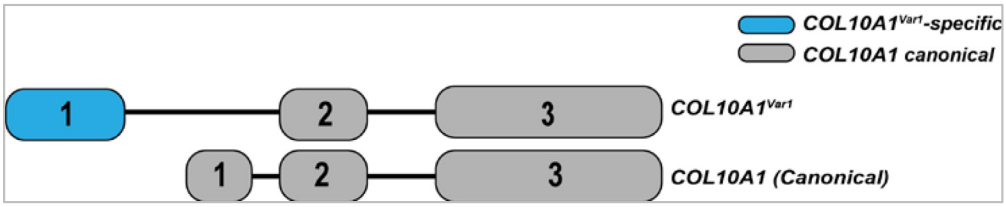
***COL10A1*^{Var1}**

Exon1: 1-236

Exon2: 237-405

Exon3: 406-3444

CDS: 252..2294



Supplementary Figure 1. Full-length structure of novel transcript-variants identified in EACs. Shown are the complete mRNA sequences (5′ to 3′) of the respective candidate transcript-variants discovered in EACs. For each of the 7 candidates, variant-specific sequences are highlighted in *blue font*. Shown below each of the sequences are positions of individual exons and coding sequence. For each of the variants and their corresponding canonical genes, exon-intron structures along with their relative sizes-distances are illustrated on the *right*.

CCNB1^{Var1} mRNA, complete (1,650 bp)

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AGACAGATGGAAATGAAGATTCTAAGAGCTTTAAACTTTGGTCTGGGTCGGCCTCTACCTTTGCACTTCCTTCGGAGAGCATCT
AAGATTGGAGAGGTTGATGTCGAGCAACATACTTTGGCCAAATACCTGATGGAACTAACTATGTTGGACTATGACATGGTGCAC
TTTCCTCCTTCTCAAATTGCAGCAGGAGCTTTTTGCTTAGCACTGAAAATTCTGGATAATGGTGAATGGGTAAGCTGTGTCCCAC
AGAACTCCTAAGCTTTTAAATTTTAAAGAGTGACTAAATACAGAACTTTTGTTATTAAAAGGCAATTCAAGTGGTTCATAAATA
TGGGATCTACTGAAGGAATAGTTAAAAAAGATGTCTTAGAATGTTTCCACATCTTTTTGTTTTTG

CCNB1^{Var1}

Exon1: 1-424

Exon2: 425-594

Exon3: 595-765

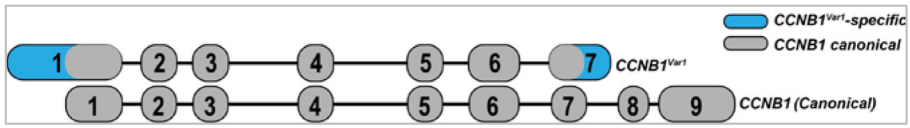
Exon4: 766-948

Exon5: 945-1107

Exon6: 1108-1344

Exon7: 1345-1650

CDS: 114..1415



Supplementary Figure 1. (*continued*).

ADNP2^{Var1} mRNA, complete (5,372 bp)

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AAGCTACATGGGTTTTAGAATCCTTACGTTTAGGCTTTGCGTACATATAGAGGATGTAGATAGTTATTTGTTGGGAGGTCTCCT
CTGTTTATTTCTTATGAATCTGTAATTTTGACTTGGATTATTTCAACCTTTTCCTCAGATTTCATACTTAAAGGGTACTCCATCAA
AACTTGCTGAGAGCTTTATAGTCTGTCCTCTGTATCCAGGGGTTCTGCATCTGTTGATTAAACCAACTGTCTGTTGAAAATATT
TGGGAGAGAAAATGCATGGTTGCATCTGTACTGAACATGTACAGACTTGTTTTCTTGTCACTGTTCTCTAAACAAGGACCTTAA
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GATAAGTTTCACATGTCTAAAATGTAACTTTTCAAACACTTTGTACTACAGCATGAAGAAGCATGTGCTGGTAGCCCATTTTCACT
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TGTGGGCACCAACATTCTGCCTGTGAATCAGCCAGTGAGACCTGGTGCTTCGCAGAACACCACCTTCCTGACATCAGGCTCTAT
TCTCAGACAGCTCATCCCTACAGGGAAACAAGTGAATGGGATTCCAACCTACACGCTGGCCCCCGTGTCTGTCACTCTGCCGG
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CAGACACAAACCAGGTGCTCAAACAGGCCAAGCAGTGGAAGACCTGCCCTGTCTGCAACGAGCTCTTTCCGTCCAACGTCTAC
CAGGTCCACATGGAGGTAGCGCATAAGCACAGCGAGTCCAAGTCTGGTGAGAAACTTGAGCCTGAAAAACTGGCAGCGTGTG
CACCATTTCTAAAGTGGATGAGAGAGAAAACGGTGCGATGTCTGTCTTGTAAGTGCTTGGTCTCTGAGGAAGAGCTTATACACC
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GGCACCTGGGGAAGAAGAAGTTGCCTATGGATTATAGCAACAGAGGTTTTCAATTAGATGTCGATGCCAATGGCAACCTGCTCT
TTCCCCACCTTGATTTCATCACCATATTGCCAAAGGAGAAGCTTGGGGAGCGGGAAGTCTACTTGGCAATCCTGGCTGGGATAC
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TCGCCGTCCATTTGGTGCGCTGCAGAAGTGCTCCCAAGGACAGCAGCTCAGACCTGCAGGCCCAGCCGGGTTTTATTCACAAC
AGTGAACTGCTTTTAGTCAGTGGTGAAGTGATGCATGATTCCAGTTTTTCTGTTAAGAGAAAGCTGCCTGACGGCCACTTAGGG
GCCGAAGACCAGCGGCATGGGGAGGAGCAGCCTCCCATCCTAAATGCCGATGCAGCCCCGGGTCCAGAAAAGGTGACGAGT
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GCTTTCCCTGTAGCAGCAGATGGTACAGTGAGTGTTCAGAGACGTGGGTACAAACCCTGTGATGTATGTATAAGGCTCCCTGA
GGATGCACTGCATTAACTTACGCTGACTTCTTTGTAAGATCTTTGCTTATAGATTATAATTTAGATCTGTATTTTTTTAGGTTTATC
CTAATAGCTGTTTTTTTTTTTAACCATAACTCATAGAAAATCAAATGTTTTTATTTGTTAAAAGTAGACTGAATTTGACATCTGGTAT
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TGTCCGGGTCGATGGGATGGCATATTGTTAAAGAATCTCCATTGTCGTCACTGTCCTGTGGATGTTAAACCTCGTTTTTTTCCCC
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GCAGAGGAACCCCTTCACTCTATCTTTAGGTAGAAATATTTGGAGGATGTGACAGCCTCTGAGAAACATAATGTTACTATGTGGA
TTTTAAAAAATATAATACTTGCATGTAATTGCTATAATGTTCATATTTGAGGCAGTTGTGAAACTGGTTTATGATTGTTGGTGTACT

Supplementary Figure 1. (*continued*).

(ADNP2^{Var1} mRNA *continued)*

CTGTTGTAAATTCAAAGAGAGCTTGTTGAACATTTTTTTTTTTTTACCTATTGTTTTCAGAGTGTCTATTTTGAATTAAAATTTGTTAC
ACCGCTGCAAATAGAACTGTTTAATTCTTTTAAAAGTTAAAACATTATTGTGAATCATAGG

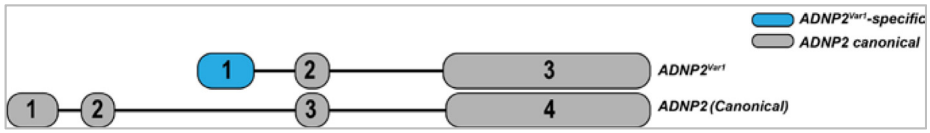
ADNP2^{Var1}

Exon1: 1-497

Exon2: 498-587

Exon3: 588-5372

CDS: 225..3620



Supplementary Figure 1. (*continued*).

SAYSD1^{Var1} mRNA, complete (14,526 bp)

TACACGCGACCCGCCAGACTTCACAAACGGAGGAGACGCGGGTCACACCAGTGCGCATGCTCAGCAGGGGCTTCTGCCAC
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ACGGACGCGAGGCGCGCGCC**ATG**GAACAGCGGTTAGCTGAGTTTCGGGCGGCGCGGAAACGGGCGGGTCTGGCGGCCCAA
CCCCCTGCTGCCAGTCAGGGCGCACAAACCCCAGGAGAGAAGGCGGAAGCAGCAGCGACTCTAAAGGCAGCCCCAGGCTGG
CTAAAGCGGTTCCTGGTATGGAAACCTAGGCCCGCGAGTGCCCGGGCCCAGCCCGGCCTAGTTCAGTTAAAAAACAATGTCTA
ACCCATGCAATGAACTTTTGACTGTATGTCTCATTTCCAAAGGAATGAGATCATTGAGGTGGACCCAGAGAAACTGAATTCAGAG
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ATTTAATTTTTTTTCTTCAGATTCTGCCTCTAGTTTAGAAAAGAAAAAGTCTAATTTACAATTCATGTCAGACAATTTTGATGGCAC
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CAACTTTAGAACGTCTCTATCACTCCAAGAAGACCCCTCTTGCACATTAGCAGTTACTACCCATTTCCAACCCCAGCCCCGCACA
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CTTTGCAGGCCTAGCATACTCTTTGTTCTCCTTCCTAGGAAATCGCTGATGGTAAGTTTTTCCACTCTTTCTTTGTTGAATTAATTT
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GGTAGTAAAAGTCAAGTCTTCATCATCTGGTTTAGGCCATGAGTCAACACACAGTTTTTTTTTAAATACTGAATGCAGATGCTTCA
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TCAGGTGTATAGGACTAAGGGCAGCTTGCGGGTTAGCTCTGTGACTGCATAGTTTTTCTACCTTCTTTCCCTGATCTTTTGCTGC
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Supplementary Figure 1. (*continued*).

(SAYSD1^{Var1} mRNA *continued)*

AAGGGAGACCTGTTTGTTTCATTTCTCATCTGTTTGGGAGATGATTTTAGAGCACTAGAAAGGCACTGGGGAGATTCTCAGCTTA
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AATGACACTGTGCTGAGTTTAAAGGAGGACTAGGTACTCCCCCAAAAAAGAAGGTGGGGGCAAAAAGTAGCATTTTGGTCA
GAGAAAACCACCTGGGAGAAAGGTGATAATCATGAAGGAGAATAACAAGCTTGGATGCATTCTGAAGACTTGGATGTTTAG
GAACAAATAAAAGGAATTGATGAGAGGCAATTCCAGAAAGGAATATTGAGTGCCTCTTGCTTTTTGAGCAAACAATATTTCTT
AATAATTACATCAGCACAAATAGAGCTGCTTCCTGCTTTTTCACAGCTACAGAGTGTACCATTGTATGGATAGATTATAACTCA
ACAAGTCTCCATTAATGTATATTTAGGTAGTTTCTAATCTTTTGTTGTTGCAAACAATGTACAACAAATATCCTTGCACATATGT
CATTTCGACATATGGGAATACTTCTCTGGGATAATGTCTTAGAATTAGATTTGCTGGCAAAAGGATATTTGACTGAAAATGTTG
ATAGATGTTGCCCTGCGTAGAAGTGATTCTTATTTACATTCCTACCAGCAATGTATGAAAATGCCAATTTCCTCATATCCTCAC

Supplementary Figure 1. (*continued*).

(*SAYSD1^{Var1}* mRNA *continued*)

CAACACAGGGTGTTTTCCCAACTTTCTGTACTTTGCCAATATCTGAGAAGTGAAATGACACCTCATAGTTTTAATTTAAATTTC
TTATTATGGGAGAACATCTTTCTATAAATTTGTGAGCCATTTGTATTTATCTGTGAACTCTTTTAGCTTTTGCGTGATTTCTGTTG
AGTTCTTGGGAATTTTAAAATTTCATTTAGGAACTCTTCATAAATGAAGAAAATTGGCTCTTTGAATTATGCATTTCAAATATTT
TTGCTCAATTTGTTCAAACTTGTTGCCGTTTTTGTTTCTGTGTGGAAATTTTTTGTTGTCAAATTTATGTATTTTATGACCTGGGT
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GAGTGGGGTTACAGATAGGGATCCAGCTTTATTTATTCAGGCATCTATTTGTTCAGGTATCTAGTTCCCTCAGGTTTATTTATT
AAATAACCCATCTCTTTCCTGCTAATTTAAAACCCTTCTGTATCATATACCACATTTCTTGTGTATTTGTATCTATTTTGGGCTTT
CTGGTCTGTTCTTTTGATCTGACAGCTTTTTCACTTTTTATTATTGTAGCTCTTTAGTGTGTTTTAAAATCTGAATGGGCTAGAC
ACCTCTTTATTACTCTTATTTTTGCTTTCTTTTTTAAAACTTACACTTAACATGTTTATTTTCTCCCTGAGTTCTTTAGAATTAACT
TGACCAGGTCCCCAAAATTTCTCTTGATATTTTCATTAGTTTCACATTAAATTTACAGATAACTTAAGGAGAAGAAGCACCTAT
AAGTTATTGTTTTACTGTTCAAGAATACGGTATGCTACCATTTGTTTAAGCCTCTTTTACTGTCCCTCAGTTGCATTTTCAAGTT
TGCTTTATATTGATCTTACTATTCCCTGTTATTCCTGGTTATTTTATCTTTTGCTATTAAGTTCAGTAGATCTTTTCTTCTATTATT
TCCTCCAACTTGTTGTTGTGTATGTGTGTATGAAAACTATTGATTTTGGTATATTAATTTTGTACCAGTCCTTTACCAAATTCTCT
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CTTATTTTACCTGTGGGGTTTTGTTTTGTTTTGTTTTGTTTGTTTTGTTTTTTGAGACAGGGTCTTAATCTTGTCACCCAGGCTGG
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TGGGACTACAAGTGTGCGCCGCTATGCCTGGCTAATTTTTTGTATTTTTAGTAAAGACGGGGCTTTGCCTTGTTGGCCAGGCT
AGTCTTGAACTCGTGGCCTCAAGTGATCCTCCCCTCTTGGCATCCCAAAGTGCTGAGATTACAGGTATGAGCCACTGTGCCC
GGCCTCTTTTCCCAATTTTTATGCCTCTTATTTTTTTCTGTTTGTCCATTACGTTGGCTAAAGCACCCAGAAAAATATTAAATCA
GAATAGTAATCAATGATATCTTTGTTTTGCTCTTGACTTTAATGAGCATGTGCCAGTATTTCCCACTAAGCATGATCCTGGCTT
TGGGGTAGTGAATAGATAGACAGACAAACAGATATCTTAAGAAATTACTCATCTGGGCCAGATGCAGTGGCTCACGCCTGTA
ATCTCAGCACTTTGGGAGGCCAAGGCAGGCAGATCACTGAAGGCCAGTAGTTTGAGACCAGCCTGGCGAACATGGAGAAAC
CCCATCTCTACTAAAAATACAAAAATTAGCCAGGTGTGGTGGTGGGTGCCTGTAATCCCAGCTATTGGGAGGCTGAGGCATG
GGAATCACTTGAACCTGGGAGGTGGAGGTTGCAGTGAGCCGAGATCGTGCCACTACACTCCAGCCTGAGCGACAGAACAAG
ACTCTGTCTCAAAAAAAAAAAAACAAACAAAAAAGAAATTACTCATCTGTACTTACGTTATTTAAAAGTTTTTCTTAAATTAAGA
ATGGATGTAGAATTTCACAAAATCTCCTTTCAATGTAGTGGAAATTAATCTTATGGTTTTTCTTTTTGATCAATTAATATAATCA
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GCTGATAGATTCTGTTTGTTAATAGTTTTATTTATGGTTTTTATCAGTGATTTTCATGAGATTGTAGTATTTTTTAGCACTTTAGA
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TCTACTGATTTATTGACTAATTGATTGACTTTGTATCTTTAAATATAATTACAACCTACTACTTGAGTTATCAGCCTTAAATGAT
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AGTACAAACCAGGCTAACCTCTTTGAAATTTAAAATTCTATATTTCCATCTCTTAGAAAAGAGTAATTAGAAGCCAATCCCTAT
TAAAACCGGATCTCACACTTTAATCACCAAATCCCCTGGTGATCTCGTGAAAATGTGGATTCTGATTAAATAGGTCTGGGTCA
AGGCCTGAGATTCTGCATTTCTAACCACCTCGTAGGTGATGCTGATGCCACTGGTCTTTGGACCACACTTTGCATAGCAAGGT
CTTTGAATACATTTCATTTCTAGGGAATTCTACTTGAATAAACAAAATGCTGGAAAACATAGTCTATAGCAGTAGTTTCACTGT
GTCTTATGATACAGTTTAAAATATCACATTTGTATCATAATAACTTGTAGCATATAATTATTATTTGTGAACATTCATTTTACAAT
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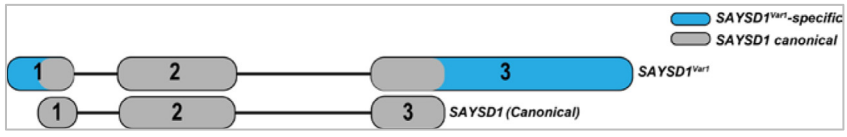
SAYSD1^{Var1}

Exon1: 1-386

Exon2: 387-4793

Exon3: 4794-14526

CDS: 4706..5056



Supplementary Figure 1. (*continued*).

DNAJA2^{Var1} mRNA, complete (1,867 bp)

ACGAAGCTGCTGCGCAGGCCTGTTAATTCCCTCAGCAGCCAGTGCGAGGTTAAACACGCGACCTCGCAACCATGGCTTTTGA
GAGGAAGCTGAATTCCTGGGGTGCGCCGTGCACAGTTCTTCATTTCCTACGTTGGCCCTGCCCAGCCGCCTGGACCTGGAG
GAAAACAGAGAACCTGGCCCACTCTTCAATACTTCGACCCCAGTGGCCGCGCCTCGGGCGCTCGGGGGCGGGCTCTGCACA
GCGACGTCACGGCGCACCGTGCGCTGGGTCAAAGTTCAGCCCCGCCCCCGCTTCCCCCTCGCTGTCTCCCTCGGCCTGTGC
CGCCGCCGACGCCGCTTGTGGGCCCGACTCCGCTCTGTCTGCTTCGCCACCTTCTCCCCGAGCACTGCCCGGCCGGCCGCC
ATGGCTAACGTGGCTGACACGAAGCTGTACGACATCCTGGGCGTCCCGCCCGGCGCCAGCGAGAACGAGCTGAAGAAGGCAT
ACAGAAAGTTAGCCAAGGAATATCATCCTGATAAGAATCCAAATGCAGGAGACAAATTTAAAGAAATAAGTTTTGCATATGAAGT
ACTATCAAATCCTGAGAAGCGTGAGTTATATGACAGATACGGAGAGCAAGGTCTTCGGGAAGGCAGCGGCGGAGGTGGTGGC
ATGGATGATATTTTCTCTCACATTTTTGGTGGGGGATTGTTCGGCTTCATGGGCAATCAGAGTAGAAGTCGAAATGGCAGAAGA
AGAGGAGAGGACATGATGCATCCACTCAAAGTATCTTTAGAAGATCTGTATAATGGCAAGACAACCAAACTACAACTTAGCAAG
AATGTGCTCTGTAGTGCATGCAGTGGCCAAGGCGGAAAGTCTGGAGCTGTCCAAAAGTGTAGTGCTTGTCGAGGTCGAGGTGT
GCGCATCATGATCAGACAGCTGGCTCCAGGGATGGTACAACAGATGCAGTCTGTGTGCTCTGATTGTAATGGAGAAGGAGAGG
TAATTAATGAAAAAGACCGCTGTAAAAAATGTGAAGGGAAGAAGGTGATTAAAGAAGTCAAGATTCTTGAAGTCCACGTAGACAA
AGGCATGAAACATGGACAGAGAATTACATTCACTGGGGAAGCAGACCAGGCCCCAGGAGTGGAACCCGGAGACATTGTTCTTT
TGCTACAGGAGAAAGAACATGAGGTATTTCAGAGAGATGGGAATGATTTGCACATGACATATAAAATAGGACTTGTTGAAGCTCT
ATGTGGATTTCAGTTCACATTTAAGCACCTTGATGGACGTCAGATTGTGGTGAAATACCCCCCTGGCAAAGTAATTGAACCAGG
GTGTGTTCGTGTAGTTCGAGGTGAAGGGATGCCGCAGTATCGTAATCCCTTTGAAAAAGGTGATCTTTACATAAAGTTTGATGTG
CAGTTTCCTGAAAACAACTGGATCAACCCAGACAAGCTTTCTGAACTAGAAGATCTTCTGCCATCTAGACCGGAAGTTCCTAACA
TAATTGGAGAAACAGAGGAGGTAGAGCTTCAGGAATTTGATAGCACTCGAGGCTCAGGAGGTGGTCAGAGGCGTGAAGCCTAT
AATGATAGCTCTGATGAAGAAAGCAGCAGCCATCATGGACCTGGAGTGCAGTGTGCCCATCAGTAAACTCTGCAAACAAATTGC
ACAGTTTATTTCTAGGTCTGCTAGAACCTGAAAACCTGAATGAAAAAAGGTGTGCTGACACTAATCAAGTCCTGTGAGGTTTAAA
TTATTGACCTATCCACTCTACCTCCATTGTACAAAAAATATTTTACAACAAGCCTGGGTAAGATTCAACAGCATAGTAGTTTTGTA
TCCAAGGTTACTTCCCCACAACCACTTTAAACA

***DNAJA2*^{Var1}**

Exon1: 1-484

Exon2: 485-544

Exon3: 545-768

Exon4: 769-849

Exon5: 850-983

Exon6: 984-1180

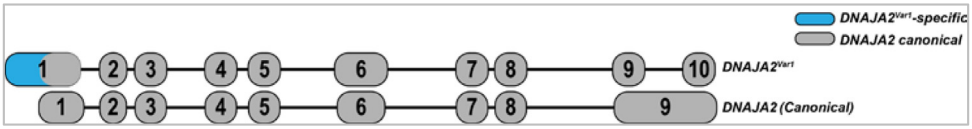
Exon7: 1181-1325

Exon8: 1326-1453

Exon9: 1454-1667

Exon10: 1668-1867

CDS: 103..1341



Supplementary Figure 1. (*continued*).

GMFB^{Var1} mRNA, complete (3,999 bp)

GTGATTTTATAACTTATCCTTGGAACCCATTTAATTTTGTGGAAAGATAATAATGAAGGTGTGGCCTAAAAGAATAGGACAGA
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CTTCATTGTGTATAGTTATAAATATCAACATGATGATGGAAGAGTTTCATATCCTCTGTGCTTTATTTTCTCCAGTCCTGTTGGAT
GTAAGCCTGAACAACAGATGATGTATGCTGGAAGTAAGAATAAGCTAGTCCAGACAGCTGAACTAACCAAGGTATTTGAAATAA
GAAATACCGAAGACCTAACTGAAGAATGGTTACGTGAGAAACTTGGATTTTTTCACTAATGTGAACTTCTGTGTTTCTAAAGTATT
TATGTATTAACCTGACCATACTGGAATCAGACATAAATACTTATTTATGCCTAAAAATGCACTGTTACTTACAGTTTGTTTCCTGCA
GTAAAGAAAAATTCTTCATTTGTGCAAAATTTGAACAAAGAGGAAATCATCTTCATAGTAATGAAACTTTGTAAAGTGTTTCCTTAT
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GCAGTTTATTTATGAAAATAGCGTATTATCACTTGTCAAATCATTGAAATTCATTTGGGGTCAAAGACTTGAGTGACCCAGTATTG
AGCCATGAATAATTTAGTGTAACCTGTATTACAAGTACATTGATGAATTCTGTATCTTCTTTGGTTTCCTGTATCTTTTTAATCAAG
TCTAGAAACTATGTTCATCAGTCACTCATTTTTAAGGTCGGGAGTTAGATTTTATGATAGAATTATGACTGTTAGCTTTTCTCCTTA
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AGCTTTAAAGTTAGTTTAAGACTTTTACACTGCCAGTATTCCACATTTGGTGAAATTAATACTTTTTTAAAGGGTCCAAATAAAATA
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GGCAGTCGAGAGTTATAATTTTGGGTATACTTGTGGTTAGTTTTGTGCCATAGGAAAAAATTATCTTAAAACTTTGGCCATAGTTA
ATAACATTAACACTTCAATAGCAATCACATCTTATATCCTAAATGTCAGAAGATATTCTGAACTGGATGCCTGAATAGTTAACTAA
ACCAGTCTTGTTAGATGATGGTACTCTTGGCATAAAGCGAGGATTCTGATATTTGGCATACTTGTAAAAACAAATACATAAGTAA
CCATTGAACATTAATTTGATAATAGGTCTAGAGACTCTAAAAACTAACCAAACTTGGTGAGTGTATTCTTATATTAAGAATATCTTA
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AGCAATAGAAAGTTGGAGATGAGGAAGTGCTAGAGTAGGTGTTTGTTTTGGTTCTTGGAGGGAAAAGATTCTTTATTCCAATTTC
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GTAGGAATGCACATGTAATCCAAGTGAGTGGAGAGTGTTTTTAATGTTTTTGAATGAAGGAAATGAGGTTTTGTTTCACCTGTTTT
GCAGCAGTAAGAGAAACTAGTGCTGCAAGAATGTATTTTTTAATGAAGTTCCTTATTTTGTCTTGCATGTTTTAGTTTTGCTTATTT
TTAAATTTGGAGGTCCTCCATAATGTCAGATAATATTGACCTGCCATACGTTAGCACTCTTAGTTCCGCTACTGTCTTTAACAGGA
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TTAGAGGATCAGTAGGACATTTTTATTCCATCTGTCCTATGGGGAAATTTACAAATCCCGTGCTCTAAAATGTTCTCAAACATTTA
TATAGATTTCCCTTTCATCTTACTAAATTTTGCATTGTTCTTTTCAAGTATGTTTCGTATTTACTGTCTTTTTTTCTGCCATTTCCCA
AATAATAACTCCAGATTTCATAATTCCAGTTTTTACATTCCGTTATCTTTCTGGTACAACCATTCCCATTCAGCCTTAAATCTGAGT
CCTTTTTAGCAGCAACTTTTTTCCTGGGATCCTCCTTCGTGGTCTTCTAAGTCAGTGTTAGTTTTGAAATTTTTGGCCCTGCATAA
GTTCTGCATAGCATCTAATGTCAAAATAGAACCAACTGGTAATCACAGTATTATTTAGTGTGGTTTCCATGACAACAAAAATACAT
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GTGTCAGCACTTTTCCCTCCTTTTTTATCTCCTATTTTCAGGAGTCAAATGTAGCCATAAACTGTATCCTTGTCTGACACTTTAGC
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CAATAGACTGATAATACTGGAATTTATAGAGTTTGAGCCATTACAACTTTTGTGAGGATGTGTTTCAAACATTTCTGGACAAATCT
TATTTTGTATTTCTGGAAGAATGTAGTAATCTTCTAGACCGCTTAAAACCAATGCTCCCAAGCTGAATATTCTTGAGAAATTTGTTT
TTATTATGCCATTTGACATTTCAAATCAGTGCTCATATACAGTAAACTTGTGATAGAAATTGTATTTTATTGCTTTTTGGATTATAAT
TCATATAAATATAATTACTTGAATATTGTTTGAGATCATTAACATGCCAGGGCAGTTCCCACTGATTTAGATGGTCCAAGATAATC
TCATTCAGGAGGCTTGAAACATTAATGGTTTAGTCTTGTGAATTTTAACAGTTCTCTGTCATCGTTTAACAAAACCAACAACTGAC
ACAACTCCTTAAGCTGTGGTTTCAGTCTCTGCTAGTTCATATTGCATGTTTATTTTGGACAGTCTTTTGTTAAGCATGGTGCTTGT
ACTGGTTTAAATAAAATGTTAACATTAAAAGAC

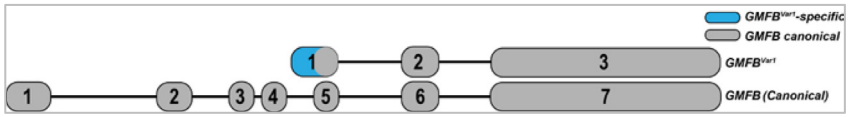
***GMFB*^{Var1}**

Exon1: 1-249

Exon2: 250-323

Exon3: 324-3999

CDS: 54..482



Supplementary Figure 1. (*continued*).

RAB11FIP5^{Var1} mRNA, complete (5,705 bp)

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GTTTCTCAGCTGCATCTGAAGCCTGGAAGCCCAGGCCGATGGGTCAGAGCAGGAGCCTTTGGGGAGAGGCTATCAGTGGCT
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GCTCCCCTCTTCTCACAGGTGGTACAAGCTGCACTCCAAGCCAGGCAAGAAGGAGAAGGAACGCGGCGAGATTGAAGTCACC
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ACCTGGGCAGCCTGGGCAAGATGGGCAAAGCCAAAGGCTTCTTCCTCCGCAACAAGCTGCGCAAGTCGTCCCTGACCCAGTC
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ACCCGCTCACCAAGCCGTAGCAGCTGGCTGTCCACTGAAGGGGGCAGGGACTCTGCACAGTCCCCCAAGCTGTTCACCCATA
AGAGGACCTACAGCGATGAGGCCAACCAGATGCGAGTGGCTCCTCCTCGGGCCCTTCTGGACCTTCAGGGCCACCTGGATGC
TGCCTCCCGCTCTTCGCTCTGTGTCAATGGGAGCCACATTTACAATGAGGAGCCCCAGGGCCCTGTGCGGCACCGCAGCTCCA
TCTCGGGCTCGCTTCCATCCTCTGGCTCCTTGCAAGCTGTCTCTTCCCGGTTCTCCGAGGAGGGGCCTCGTTCCACAGATGAC
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TGGCCCCTGGGGCCAGCCACCCTGGAGAGGAGGAGGGGGCCCGGCTACCAGAGGGCAAGCCAGTCCAGGTTGCCACACCC
ATAGTGGCCTCCTCTGAGGCTGTGGCAGAGAAGGAGGGAGCCCGGAAGGAGGAACGCAAGCCCCGGATGGGTCTCTTCCAC
CACCACCACCAAGGCCTAAGTCGGAGCGAGTTGGGTCGCCGAAGCTCTCTGGGGGAAAAGGGGGGTCCCATCCTGGGGGCC
TCCCCACATCACTCATCCAGTGGGGAGGAAAAGGCCAAGAGTAGCTGGTTTGGCTTGAGAGAAGCCAAGGACCCGACTCAGAA
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CTTTGCTGCCAGCAGGCTGCGTCCAGAGGCCAGGAGCGAGATCCTGGCCCCTGCAGGAGTGGGGCTGGAGGCGGCAGGGC
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AGCGCGAGCGCGGCTGACCCAGGGCTCCTCGGGTCGGTAGGGGCTGGCCTGCCCTCCTCGTCAGCCCAGCTACAGCTGAG
AGCCTCAGGCTCAGAACCAGACAGGGAACTGCCAGCCCCGGAAGTGGAAGCAGGGCAGAGTCCGGCAGACAGTGGGACA
TCTCTATTTAGCTCCCCAGAAGTGATCAGTGTGTGGGAGAGGCTGCCGGGCCCAGAGAGCGCTGCTGAGGGCCAGGACGAT
GAGTCCTCCCGAGGCGAAAATCAGCTTTGCCCTGACGTCGAGACAGCTGATGATGCCTGGCCTTGGGATGTGGTCACCATTT
CTCCTGCAGCTGAGACAGCCTCACTAGTCTTTCGGGGAGAGTCTGATGAGCCTGCTCCCCAGGTGCAGCCTGAATCACCAG
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GCCCCCATACCCTGTCACTCAAAGAGCTTGGCTCTTCAGAGTCAGCACATCTGGGGGACTCCAGAGGTTGGAGAAGGCCCT
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TCCCGCCTTGGGCCAGCCACCACCGTGGGGGGCCCAGCCCTCCATGCTCTCCCCTGTCTGAAGCCTGGCCCCTGACTACCT
CCTCTGCACCACCAGGGGAGCCAGCCTTACTCCCTGGCCCCCATGAGCCCTCCCCACCTGGGGGCTCCCCTGCCCTACTTA
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CATCGTCTGTCTTGCCTTGTCACTCCTTGCTCCTCTCCCTCCTGCCTCAACTGGGGCTGCTGGAAGGGGGCCCTCTGGATTCC
CTTTGGAGGCATCAGGTTCTGTGCACAACTTGTGTGTCTTTGGGAGCCCTGGGTCTTTCCCACCTGCCTGTTTTCATATCCCTC
AGCTTTGGGGGCCCCAGGGAATTGGAACTCTTCCCCCAAGACAGAGCAGGGGTGAACTGTAGCTTGATGCAGTAATCTGGGAC
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TCCCTCCAGTCCCTGGAGGGGCTTCTAGTATTACTGGGACAATGACCACGCTGCCTGTTTGTCTGTGAGTTACGGGCAACCAG
CCTCTTCAGCCTCACACCCATTCCCCTGAGAGCAAGAAGCCTGTGTGGTCTGGGCCAGTCTCTGCCATGTCCTGAGTCTGCTT
CAGTCTGGAGCTGTTTGTGGGGCGAGTGCCATGTGGACAGTGGTGGATGATGTGTGTGCTTCAGGCTGCTCCCTGACCCCTCT
GACCTTTCCACGAGTGTCACATGGGAATGTGTGGGGCGCAGGGGCGGGTGCGGAGAGAGCACCTTTTTGCTTTTCGAGCTCTT
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GGCCCTGGGAATGGGAATGGACAGCACTGCTGTGGGCTGTTCCCCTCCCCTAAAGTTAATCTCTTGGTCTGGCCAAGTTGCTG
CTCCCTCAACCTTCCTGCTGTCTTCCCCTCCCTCAACCCCAATAGGAGGATCCCAGGATAAACACTGCTGGGCAGGCGGGCAG
GCAGGCCTGGGGCTGCCCTGCTCACTCTCATTGTCTGGCCTCAGGACTTAGCCATACTAGACCAGTCAGCTTGCCTGGAAGAG
GGAGGTCCCACTATGCCTTTGGGAGACACCTATACTTAGGAAAAAGCCTTTGTTGTCCTCCCATCCATCCATTAAGCTGCTATCT
CAGCCTGTCCCTTCTGCCCCAGGGGCTTGCCTGGCTTGGCTGCAGTGCACTTTGAAATGAAGTATCTGTCCTTTGGCCCAGCC
CCTGGTTTGCTTGTAGAAAACATGGTAGGCTTCCCCAAGGCATCTGCAGGGAACTTTGGCAGCTTGGGGCACCCTGAATTAGC
AAAAATGGGGGGTGATGAGGTGCTGAAGAAGGATACTTAACAGCTTAGTGAGGAGGCAAGAGCTCCTCTGGGACCACCACTTC

Supplementary Figure 1. (*continued*).

(*RAB11FIP5^{Var1}* mRNA *continued*)

TTCAGGAGAGGGCCTGTGGGCTTGCTTTTGGAAGGCCTCAGGCAGACACGTGCCCTCTGGGTGATGTCTGTCTGCTGCCAGG
ATGGAGCAGAGGAGCGCCACACATGGAGGAAAGCCCCTGTAACGTTACCTACCTTAAACTCCACTCATCAAATCTGAGAAAAGT
ATCCACTGGTCCCCAGGGTTTCAGTCATGCTTTTGGGGGTCATTGGGTATTAGAGAAGTAAGTATCTTTTCTGAGAGAGGGGGA
GTCACCCCCCCTACTGGGGATTCCTCTGGGCTTTATTCACTCCCAGCCCTGGCCCTGACCTTTGTGGGCCTCCCTAATGCCCA
GGGCATGGATGGCTTCAGAGGAGTTTTTGAATCGAAGCCCAGGGTCCTTGTTGATGTTTCTTCTCCTAGCCACACTTGGAGGAA
AGTTGCAGGTGGGTTGGGCAGGGAGCAGGCATGGTTCTGCTTTGCTGTTTGTCTTCCTAGTTAAGGCTCTTTATAAAGAGCTTG
TTCTTCATGTTTTAAGCACTTTATGAAGAATAAAACATTCATGTACTGCA

RAB11FIP5^{Var1}

Exon1: 1-777

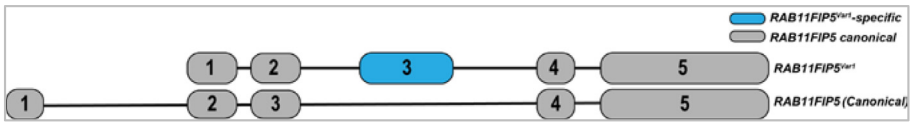
Exon2: 778-1477

Exon3: 1478-3172

Exon4: 3173-3362

Exon5: 3363-5705

CDS: 172..2133



Supplementary Figure 1. (*continued*).

Supplementary Table 1. Discovery and Validation Sample Cohorts

Discovery RNAseq samples	Number of samples	Median age at diagnosis, *y* (range)	Gender distribution	Cancer stage distribution
EAC[a]	49	65 (36 - 88)	89% (male) 11% (female)	Stage I (17.9%), Stage II (19.6%), Stage III (46.4%), Stage IV (16.1%)
Nondysplastic stable Barrett's esophagus[b]	18	56 (18-84)	94% (male) 6% (female)	NA
Normal esophageal squamous (SQ)[c]	11	64 (45-83)	90% (male) 10% (female)	NA
Normal gastric (GAST)	11	63 (36-82)	82% (male) 18% (female)	NA
Total	**89**			

Validation samples	Number of samples	Median age at diagnosis, *y* (range)	Gender distribution	Cancer stage distribution
EAC[d]	170	64 (34–89)	77% (male) 15% (female)	Stage I (14.1%), Stage II (16.8%), Stage III (52.2%), Stage IV (15.0%)
Normal esophageal squamous (SQ)	465	64 (34 –89)	77% (male) 15% (female)	NA
Barrett's metaplasia (BM)[e]	123	65.5 (36–93)	71% (male) 34% (female)	NA
BM with high-grade dysplasia (HGD)	60	66 (46–80)	89% (male) 11% (female)	NA
Normal gastric (GAST)	14	63 (36–82)	85% (male) 15% (female)	NA
Total	**832**			

[a]11% of EACs were gastroesophageal junctional adenocarcinomas.
[b]Median surveillance of 9 years, ranging from 6 to 22 years.
[c]Each of the 11 normal SQ samples was obtained from respective EAC patients included in the RNA sequencing.
[d]13% of EACs were gastroesophageal junctional adenocarcinomas.
[e]Clinical follow-up information unavailable (progression status unknown) for these patients.

Supplementary Table 2. Candidate Novel Transcript-Variants

Transcript_ variant	CHR	Transcript_ variant Genomic START (hg19)	Transcript_ variant Genomic END (hg19)	Transcript_ variant STRAND	Transcript_ variant EXON NUMBER	Transcript_ variant EXON SIZE (bp)	Transcript_ variant LENGTH (bp)	Transcript_ variant PREDICTED CDS START-STOP (bp) [a]	Transcript_ variant PREDICTED PROTEIN LENGTH (AA)[a]
COL10A1^{Var1}	chr6	116440086	116443124	–	3	3038	3442	252..2294	680
	chr6	116446502	116446670	–	2	168			
	chr6	116479777	116480013	–	1	236			
SAYSD1^{Var1}	chr6	39063820	39073552	–	3	9732	14523	4788..5138	116
	chr6	39077090	39081496	–	2	4406			
	chr6	39082659	39083044	–	1	385			
CCNB1^{Var1}	chr5	68462688	68463110	+	1	422	1643	403..1512	369
	chr5	68463735	68463905	+	2	170			
	chr5	68464000	68464170	+	3	170			
	chr5	68467097	68467279	+	4	182			
	chr5	68470078	68470236	+	5	158			
	chr5	68470704	68470940	+	6	236			
	chr5	68471224	68471529	+	7	305			
RAB11FIP5^{Var1}	chr2	73300510	73302852	–	5	2342	5700	144..3566	1140
	chr2	73303121	73303310	–	4	189			
	chr2	73306779	73308473	–	3	1694			
	chr2	73315178	73315877	–	2	699			
	chr2	73316007	73316783	–	1	776			
ADNP2^{Var1}	chr18	77889764	77890260	+	1	496	5369	765..3785	1006
	chr18	77890986	77891075	+	2	89			
	chr18	77893495	77898279	+	3	4784			
DNAJA2^{Var1}	chr16	46989335	46989534	–	10	199	1857	407..1645	412
	chr16	46990919	46991132	–	9	213			
	chr16	46992915	46993042	–	8	127			
	chr16	46993187	46993331	–	7	144			
	chr16	46998523	46998719	–	6	196			
	chr16	47001425	47001558	–	5	133			
	chr16	47001996	47002076	–	4	80			
	chr16	47005261	47005484	–	3	223			
	chr16	47005808	47005867	–	2	59			
	chr16	47007406	47007889	–	1	483			
GMFB^{Var1}	chr14	54941202	54944877	–	3	3675	3996	270..395	41
	chr14	54946504	54946577	–	2	73			
	chr14	54947592	54947840	–	1	248			

[a]Putative candidate transcript–variant coding regions were predicted using NCBI ORF finder. Listed are only those predicted ORFs for transcript–variants that are in the same reading frame as respective canonical transcripts.

Supplementary Table 2. Continued

Transcript_ variant– specific Forward_ primer (5' to 3')	Transcript_ variant-specific Reverse_ primer (5' to 3')	PCR_ product_ size (bp)	Canonical_ Gene symbol	Canonical_ Gene ID	Canonical_ Transcript NUCLEOTIDE ID	Canonical_ Transcript LENGTH (bp)	Canonical_ Transcript CDS_START-STOP (bp)	Canonical_ Transcript PROTEIN ID	Canonical_ Transcript PROTEIN LENGTH (AA)
AGCAGCC AACAACAA GCATA	GTGGACCA GGAGTAC CTTGC	252	**COL10A1**	1300	NM_000493	3302	96..2138	NP_000484	680
CATCCTC CTTCCCAC TACCA	TGCCA TCATTACA TGCACCT	2241	**SAYSD1**	55776	NM_001304793	6425	4706..5056	NP_001291722	116
AGAGG CAGACCA CGTGAGAG	GCTTA GGAGTT CTGTGG GACA	1431	**CCNB1**	891	NM_031966	2029	114..1415	NP_114172	433
TTGGCTC TTCAGAGT CAGCA	GGTAG TACTTG GCCGA CTGG	778	**RAB11FIP5**	26056	NM_015470	4272	172..2133	NP_056285	653
CCATC AAAACTTG CTGAGAGC	GGCCAC AACAGTA TGGCTTT	288	**ADNP2**	22850	NM_014913	5157	225..3620	NP_055728	1131
GGATGC CGCAGTA TCGTAAT	TTGTGGG GAAGTAA CCTTGG	503	**DNAJA2**	10294	NM_005880	3008	103..1341	NP_005871	412
TCCCCAG GTGTTG GTAAAT	GGTCTTC GGTATTTC TTATTTCAA	250	**GMFB**	2764	NM_004124	4085	54..482	NP_004115	142

Supplementary Table 3. Expression Status of COL10A1^{Var1} and Canonical COL10A1 Across Lesions

	EAC (N = 219)[a]	
	Canonical COL10A1-positive	Canonical COL10A1-negative
COL10A1^{Var1}-positive	53 (24.2%)	79 (36.07%)
COL10A1^{Var1}-negative	1 (0.46%)	86 (39.27%)
	NDBE (N = 141)[a]	
	Canonical COL10A1-positive	Canonical COL10A1-negative
COL10A1^{Var1}-positive	0 (0%)	2 (1.42%)
COL10A1^{Var1}-negative	22 (15.6%)	117 (82.98%)
	HGD (N = 60)[a]	
	Canonical COL10A1-positive	Canonical COL10A1-negative
COL10A1^{Var1}-positive	1 (1.67%)	1 (1.67%)
COL10A1^{Var1}-negative	5 (8.33%)	53 (88.33%)
	SQ (N = 476)[a]	
	Canonical COL10A1-positive	Canonical COL10A1-negative
COL10A1^{Var1}-positive	9 (1.89%)	21 (4.41%)
COL10A1^{Var1}-negative	67 (14.08%)	379 (79.62%)
	GAST (N= 25)[a]	
	Canonical COL10A1-positive	Canonical COL10A1-negative
COL10A1^{Var1}-positive	0 (0%)	1 (4%)
COL10A1^{Var1}-negative	9 (36%)	15 (60%)

NDBE, nondysplastic Barrett's esophagus.
[a]Number of samples combined from both Discovery and Validation cohorts.