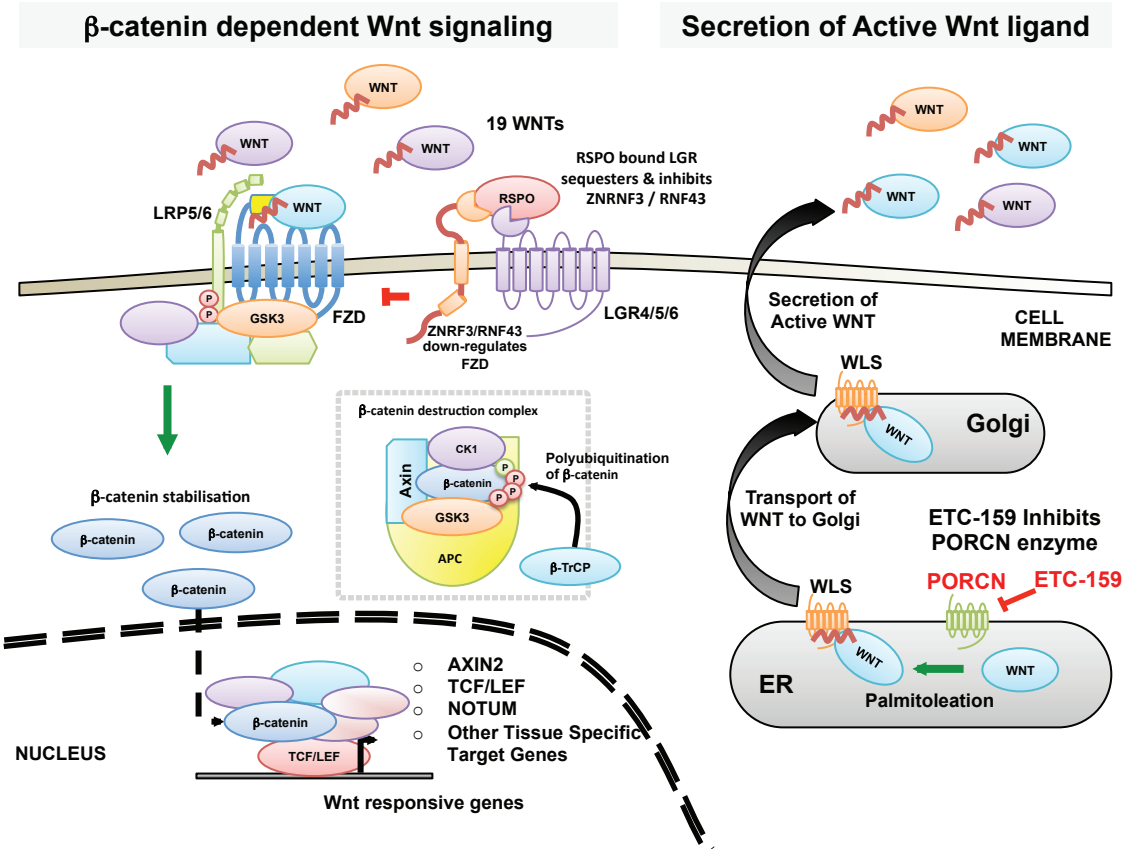
Idris et al.
SUPPLEMENTARY FIGURE S1



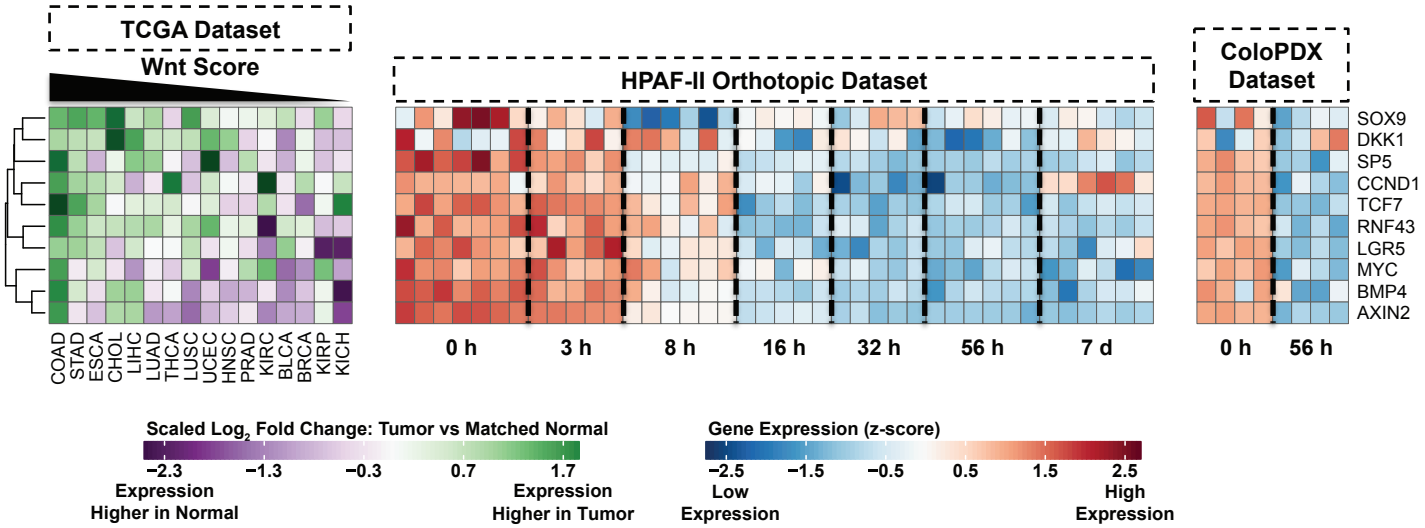
Supplementary Figure S1: Schematic illustration of the β-catenin dependent Wnt signaling pathway and the mechanism that governs Wnt ligand secretion. The binding of Wnt ligands to the FZD-LRP receptor disengages the β-catenin destruction complex leading to increased β-catenin protein abundance. β-catenin can translocate into the nucleus to bind to the TCF/LEF transcription factors and recruit other coactivators to induce the expression of Wnt responsive genes such as *AXIN2* and *NOTUM*. Note that the palmitoleation of the Wnt ligand by the PORCN enzyme (which is inhibited by the chemical ETC-159) is required for its binding to WLS (to facilitate the secretion of the Wnt ligand) and its interaction with the FZD receptor. The FZD receptor abundance is down-regulated by the membrane-bound E3 ubiquitin ligase, RNF43/ ZNRNF3. RSPO ligand binds to the LGR receptor to form a complex that sequesters the RNF43/ZNRNF3 enzyme and suppresses its activity.

SUPPLEMENTARY FIGURE S2

Human Gene	Forward Primer	Reverse Primer
EPN1 (Reference)	CTCTGACTTTGACCGACTCC	TGACCCCACTCATGTCAAAC
CTNNB1	AGCTTCCAGACACGCTATCAT	CGGTACAACGAGCTGTTTCTAC
AXIN2	CTGGCTCCAGAAGATCACAAAG	CATCCTCCCAGATCTCCTCAAA
HNRNPA1	TCAGAGTCTCCTAAAGAGCCC	ACCTTGTGTGGCCTTGCAT
HNRNPA2B1	ATTGATGGGAGAGTAGTTGAGCC	AATTCCGCCAACAAACAGCTT
HNRNPM	GCGGCGACGGAGATCAAAA	CTCATTCTGAGCAGGTCGTTC
NOTUM	CTTCATGGCGCAAGTCAAGAG	CGAGGTGTTGAGTAGGAGGTG
SRSF1	CCGCAGGGAACAACGATTG	GCCGTATTTGTAGAACACGTCCT
SRSF3	TGGCAACAAGACGGAATTGGA	CAAAGCCGGGTGGGTTTCTA
SRPK1	ATGGAGCGGAAAGTGCTTG	GAGCCTCGGTGCTGAGTTT
SRI Constitutive Exon 4 to Exon 6	GGCATTGCTGGAGGATACAAAC	CCCATTGTGCCAGACATATCTC
SRI Exon 1 to Exon 3	CCAGCTTCTACCTATAAGACTTCAACTC	GTCCAGCTACAGCAGCAAAG
SRI Exon 2 to Exon 3	ATGGCGTACCCGGGGCATC	GTCCAGCTACAGCAGCAAAG
CD46 Constitutive Exon 2	ACCAACATTTGAAGCTATGGAGC	GCCATGTATGATTCCGATCACAA
CD46 Exon 8 to Exon 10	CGACTTCTTCCACTACAAAATCTCC	CTGTCAAGTATTCCTTCCTCAGG
RECQL5 Constitutive Exon 7	GTTTGTCGCCCATTGGAATATTGCC	CTTCCTTCCTGATCAGGAAGCTGAC
RECQL5α Exon 7 to Exon 8.2	GTCAGCTTCCTGATCAGGAAGGAAG	CTTTGCTCCACATATAAAATAAGTCACTTACC
RECQL5γ Exon 7 to Exon 8.3	GTCAGCTTCCTGATCAGGAAGGAAG	CTGACTACACCAAGCAGCCCTCAGACTC
RECQL5β Exon 9 to Exon 9	CGCTGCCTGCCTGCGCCAAAG	GCTGAAGTCCCCGTAGCCCTTGCG

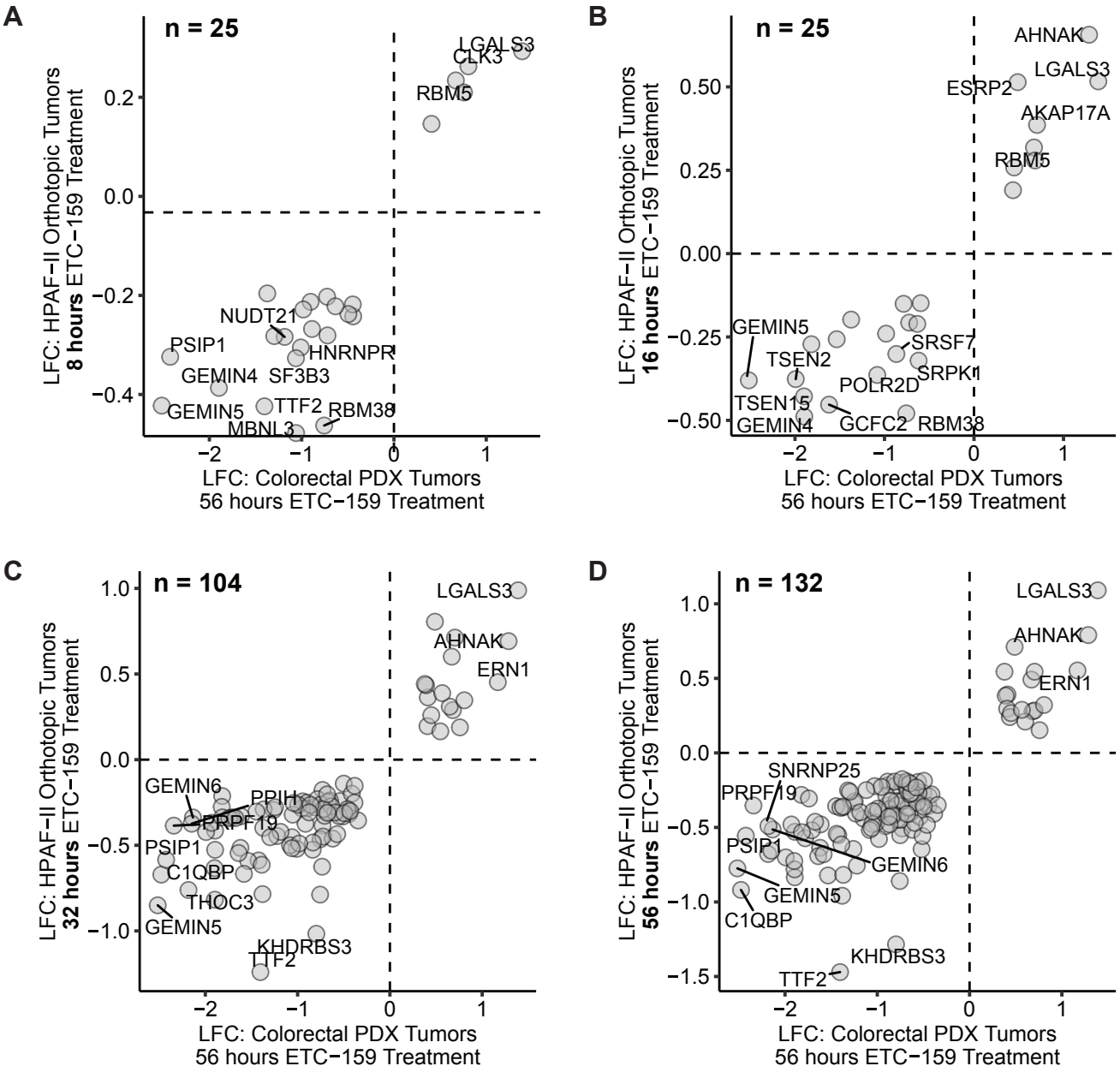
Supplementary Figure S2: Table of primers used for *in vitro* validation of Wnt regulated gene and isoform expression.

SUPPLEMENTARY FIGURE S3



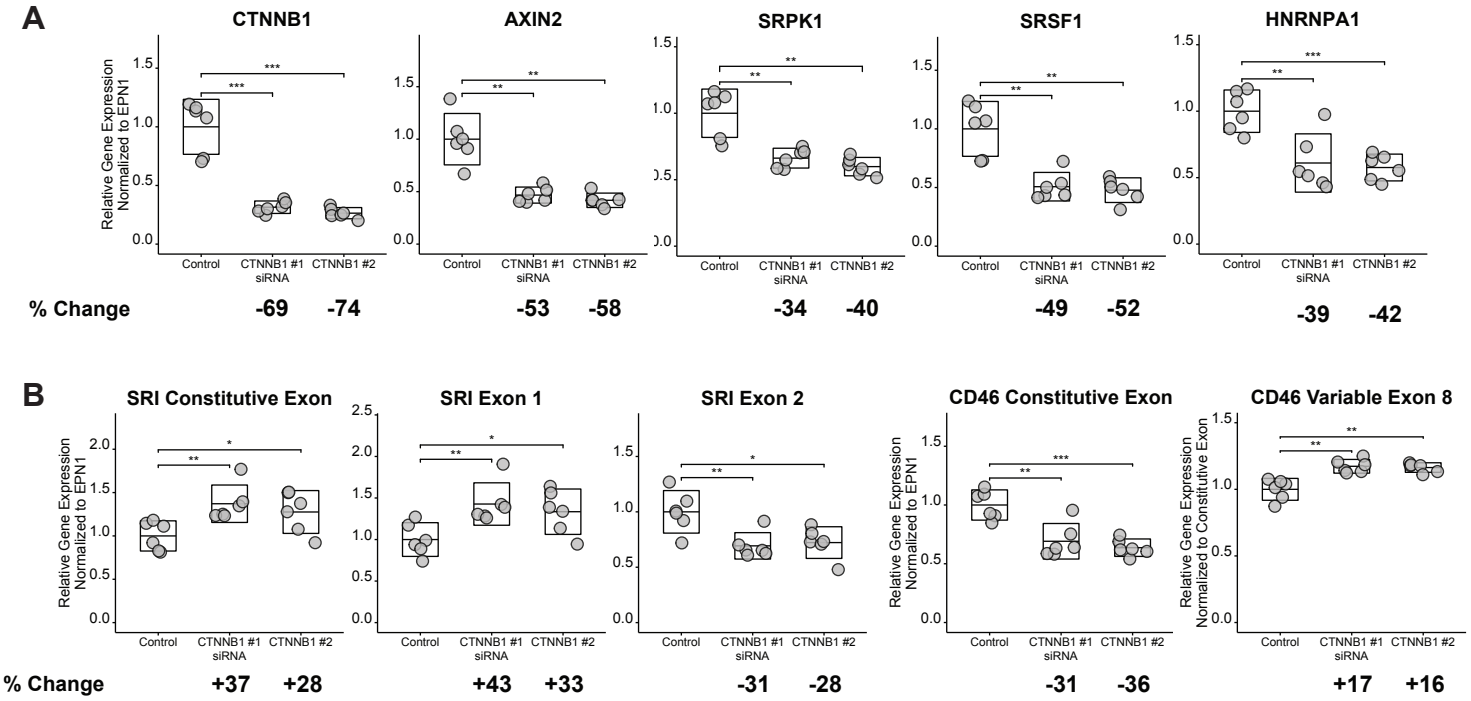
Supplementary Figure S3: Wnt score was calculated to sort the tumor types extracted from The Cancer Genome Atlas (TCGA), as seen in main Figure 4. Tumors with high a Wnt score indicate strong influence of Wnt/β-catenin signaling. The Wnt score was calculated based on the gene expression of 10 well-established Wnt target genes (shown in the heatmap). The rightmost and center heatmaps show the expression of these Wnt target genes in the colorectal PDX (ColoPDX) dataset and the HPAF-II orthotopic dataset respectively. For these heatmaps, red indicates high expression while blue indicates low expression. The expression of these Wnt target genes decreases with ETC-159 treatment in both of the *in vivo* datasets. The leftmost heatmap shows the gene expression fold change (of the tumors relative to the matched normal samples) for the tumors shown in the TCGA dataset for the indicated Wnt target genes. A positive fold change (shown in green) indicates that the expression of the gene is higher in the tumors relative to the normals. A negative fold change (shown in purple) indicates that the expression of the gene is higher in the normal samples relative to the tumors.

SUPPLEMENTARY FIGURE S4



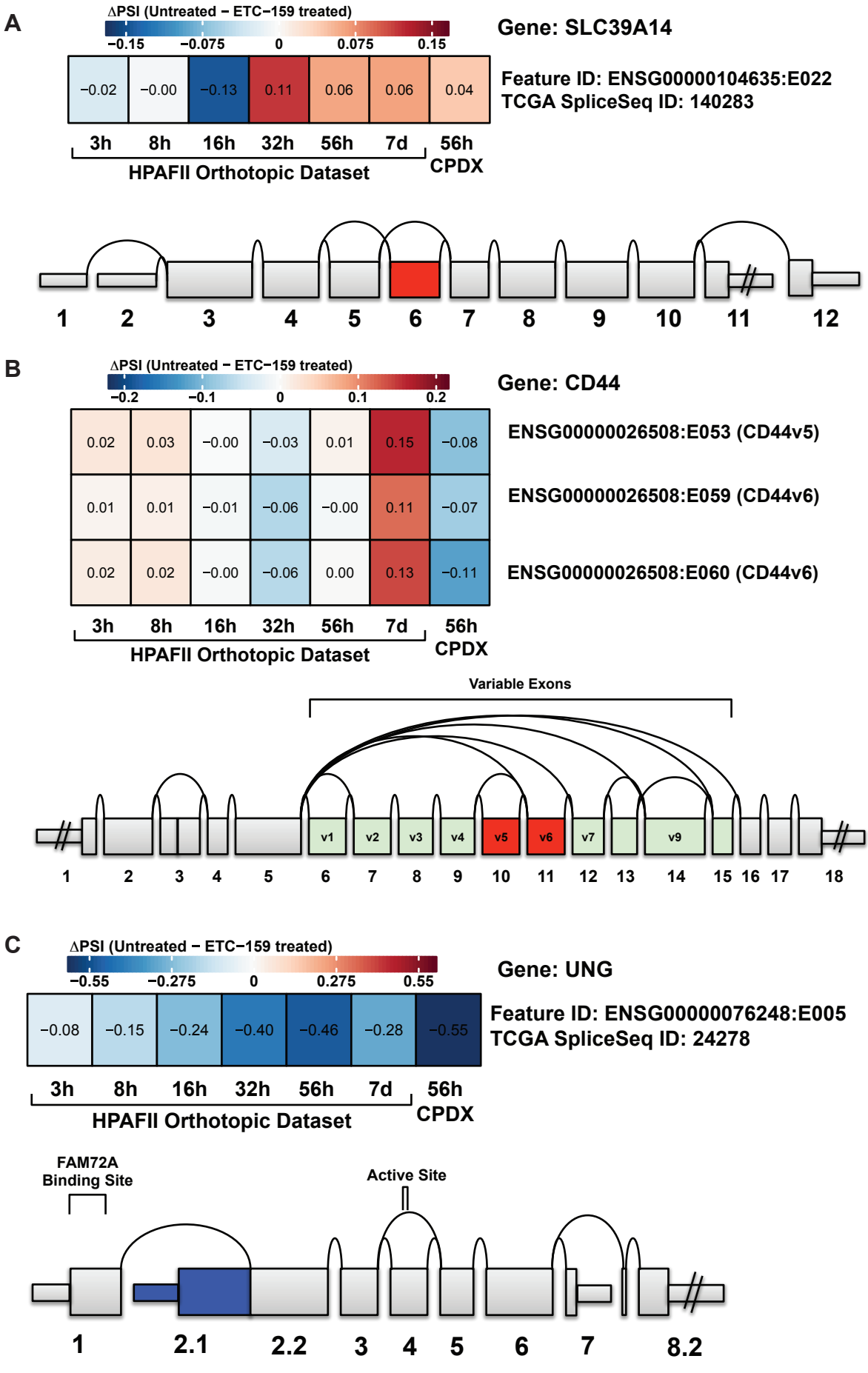
Supplementary Figure S4: 148 splicing regulators had consistent ETC-159 mediated gene expression changes in the colorectal PDX tumor model and at least one of the time points in the HPAF-II orthotopic tumors treated with ETC-159. The splicing regulators with similar expression patterns (measured in log2 fold changes (LFC)) in the colorectal PDX tumors and HPAF-II orthotopic tumors treated with ETC-159 for **(A)** 8 hours, **(B)** 16 hours, **(C)** 32 hours, **(D)** 56 hours and **(E)** 7 days, are shown. Note that there were no statistically significant changes in the expression of the splicing regulators in tumors treated with ETC-159 for 3 hours relative to the untreated controls.

SUPPLEMENTARY FIGURE S5



Supplementary Figure S5: Gene expression changes driven by the siRNA-mediated knockdown of β-catenin using 6 biological replicates. HPAF-II cells were transfected with 25 nM of non-targeting control (Dharmcon; D-001810-10) or two independent β-catenin (siRNA #1: GCGUUUGGCUGAACCAUCA; siRNA #2: GGUACGAGCUGCUAUGUUC) siRNAs using the Dharmacon Reagent according to the manufacturer's protocol. After 3 days, RNA was extracted from the cells using the RNeasy MiniKit (Qiagen) and quantitative PCR (qPCR) was performed using the primers listed in the table in Supplementary Figure S2. Both of the siRNAs for β-catenin were able to significantly suppress the gene expression of β-catenin and, consequently, the Wnt target genes, *AXIN2* and *NOTUM*. A two-tailed t-test was performed between the indicated samples. $p > 0.05$: ns; $p \leq 0.05$: *; $p \leq 0.01$: **; $p \leq 0.001$: ***; $p \leq 0.0001$: ****.

SUPPLEMENTARY FIGURE S6



Supplementary Figure S6: Schematic illustrations of the gene structure and the effect of ETC-159 treatment on the variable exon expression induced in the two *in vivo* tumor models (shown in the heatmap). The heatmaps show the magnitude of the variable exon expression (measure in ΔPSI). A positive ΔPSI value (shown in red) suggests that Wnt signaling promotes the expression of the variable exon while a negative ΔPSI value (shown in blue) suggests that Wnt signaling suppresses the expression of the variable exon. **(A)** Wnt signaling induces the expression of mutually exclusive exon 6 of *SLC39A14*. This splicing event and its association with Wnt signaling have been previously established. **(B)** Wnt signaling induces the expression of *CD44* isoforms that include variable exons 5 and 6. This splicing event and its association with Wnt signaling have been previously established. **(C)** Wnt signaling suppresses transcription of *UNG* originating at a promoter proximal to exon 2.1. The connection between Wnt signaling and this alternative promoter usage event in *UNG* was not previously established.

SUPPLEMENTARY FIGURE S7

Gene	Splice ID for our study	Time Point (ETC-159 Treatment)	Splice ID for Shinde et al.
DEDD2	ENSG00000160570:E021	8 hours	ENSMUSG00000054499:25219764-25219859:target
TSC22D1	ENSG00000102804:E020		ENSMUSG00000022010:76438933-76439108:target
CUEDC1	ENSG00000180891:E010	32 hours	ENSMUSG00000018378:88188783-88188850:source
E2F4	ENSG00000205250:E015		ENSMUSG00000014859:105298072-105298182:source
TLCD1	ENSG00000160606:E010		ENSMUSG00000019437:78179273-78179452:target
TSC22D1	ENSG00000102804:E020		ENSMUSG00000022010:76438933-76439108:target
PRPF39	ENSG00000185246:E016	56 hours	ENSMUSG00000035597:65044006-65044124:target
RGS3	ENSG00000138835:E051		ENSMUSG00000059810:62689499-62690626:target
ABR	ENSG00000159842:E086	7 days	ENSMUSG00000017631:76508888-76509419:source
PARP9	ENSG00000138496:E018		ENSMUSG00000022906:35943369-35943805:source
RGS3	ENSG00000138835:E051		ENSMUSG00000059810:62689499-62690626:target
SNRNP70	ENSG00000104852:E022		ENSMUSG00000063511:45381224-45381234:target
ZDHHC4	ENSG00000136247:E009		ENSMUSG00000001844:143328295-143328570:source
TACC2	ENSG00000138162:E018	Colorectal PDX	ENSMUSG00000030852:130674602-130674739:target

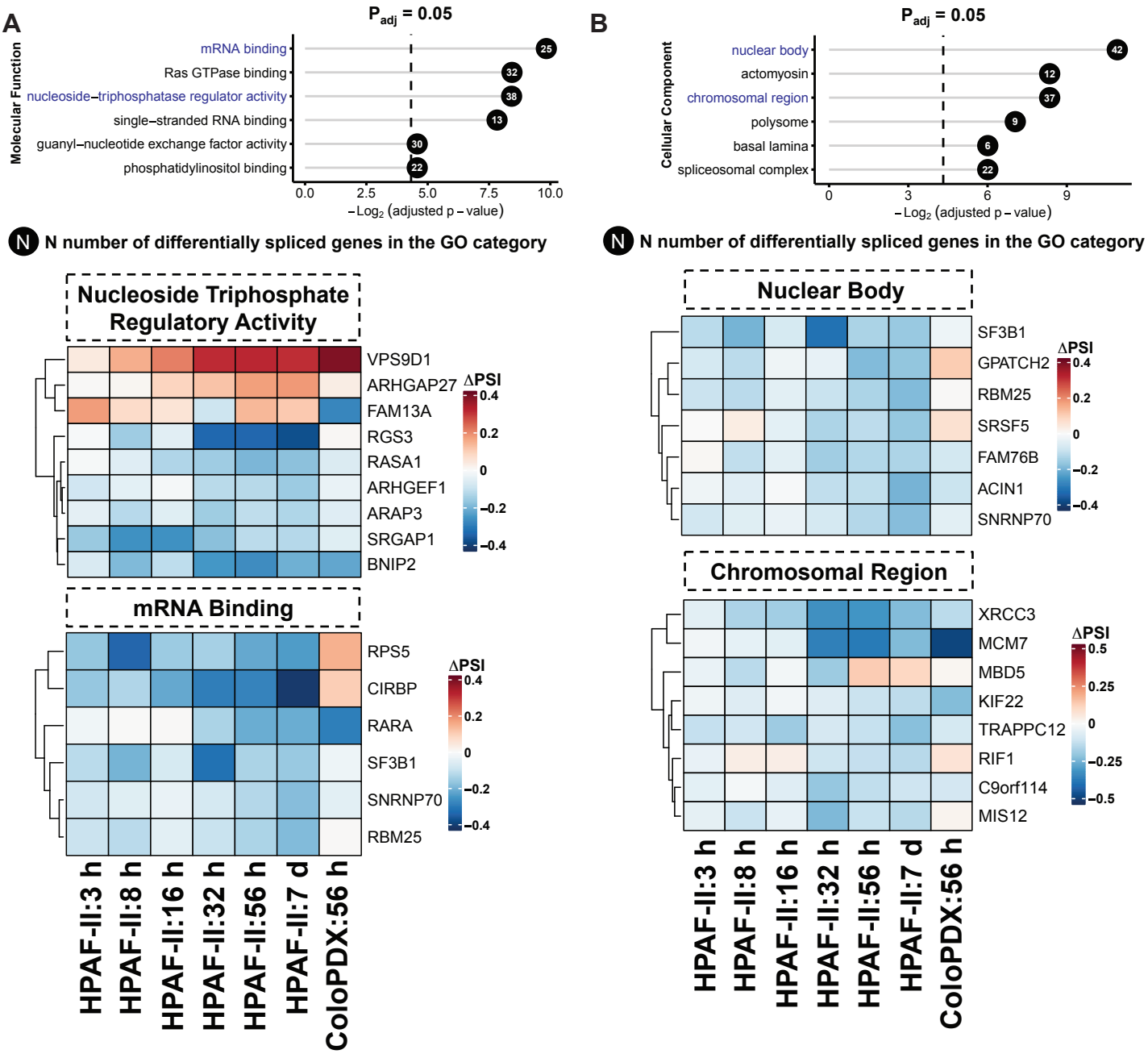
Supplementary Figure S7: A tabulation of the isoform expression changes that were detected in our analysis from the two *in vivo* human tumor models and the analysis by Shinde and colleagues with a *GSK3* double-knockout model in mouse embryonic stem cells.

SUPPLEMENTARY FIGURE S8

featureID	GeneID	Symbol	ΔPSI (Untreated Samples - ETC-159 Treated Samples						
			HPAF-II Orthotopic Dataset						Colorectal PDX Dataset
			3 hours	8 hours	16 hours	32 hours	56 hours	7 days	56 hours
ENSG00000078304:E021	ENSG00000078304	PPP2R5C	4	7	0	15	16	8	15
ENSG00000091436:E021	ENSG00000091436	MAP3K20	14	11	12	17	16	20	28
ENSG00000091436:E022	ENSG00000091436	MAP3K20	11	6	8	9	13	16	24
ENSG00000092978:E011	ENSG00000092978	GPATCH2	3	0	3	10	5	20	29
ENSG00000111652:E026	ENSG00000111652	COPS7A	4	1	0	8	13	6	18
ENSG00000118564:E035	ENSG00000118564	FBXL5	0	0	-1	10	4	10	11
ENSG00000138434:E009	ENSG00000138434	SSFA2	4	0	5	-2	9	11	18
ENSG00000138434:E010	ENSG00000138434	SSFA2	5	-1	6	-2	12	11	20
ENSG00000138434:E013	ENSG00000138434	SSFA2	4	-5	6	-10	9	11	28
ENSG00000144560:E026	ENSG00000144560	VGLL4	5	10	6	16	21	20	14
ENSG00000152767:E070	ENSG00000152767	FARP1	0	2	4	0	12	11	15
ENSG00000153944:E049	ENSG00000153944	MSI2	1	-3	4	1	14	11	14
ENSG00000154639:E013	ENSG00000154639	CXADR	7	5	2	7	14	19	16
ENSG00000160218:E008	ENSG00000160218	TRAPPC10	1	-16	3	-7	11	7	20
ENSG00000178177:E005	ENSG00000178177	LCORL	-1	10	8	11	9	12	22
ENSG00000079616:E033	ENSG00000079616	KIF22	-2	-7	-2	-6	-11	-13	-22
ENSG00000089177:E006	ENSG00000089177	KIF16B	-1	-2	8	-19	-9	-9	-18
ENSG00000094914:E043	ENSG00000094914	AAAS	-1	-10	-5	-22	-18	-14	-24
ENSG00000100105:E009	ENSG00000100105	PATZ1	-4	-12	-6	-20	-13	-10	-20
ENSG00000101158:E039	ENSG00000101158	NELFCD	-6	-10	-5	-5	-4	-5	-17
ENSG00000104129:E005	ENSG00000104129	DNAJC17	-2	1	-7	-5	-10	-9	-11
ENSG00000108469:E040	ENSG00000108469	RECQL5	0	-4	-3	-15	-14	-12	-35
ENSG00000112081:E011	ENSG00000112081	SRSF3	-1	-4	-4	-8	-10	-8	-21
ENSG00000112081:E012	ENSG00000112081	SRSF3	-2	-4	-4	-11	-13	-10	-27
ENSG00000131389:E016	ENSG00000131389	SLC6A6	-2	-14	-6	-18	0	-2	-12
ENSG00000133703:E004	ENSG00000133703	KRAS	1	-2	3	-8	-12	-14	-18
ENSG00000138600:E010	ENSG00000138600	SPPL2A	-4	-1	6	-5	-6	-10	-15
ENSG00000156787:E025	ENSG00000156787	TBC1D31	-8	-15	-8	-17	-4	-24	-53
ENSG00000166508:E033	ENSG00000166508	MCM7	-1	-2	-3	-19	-14	-12	-29
ENSG00000166508:E034	ENSG00000166508	MCM7	-2	-4	-6	-34	-35	-22	-46
ENSG00000166851:E009	ENSG00000166851	PLK1	-1	-3	-2	-5	-15	-7	-58
ENSG00000168056:E053	ENSG00000168056	LTBP3	-7	-14	-14	-24	-15	-12	-17
ENSG00000172167:E019	ENSG00000172167	MTBP	-5	-3	-5	-13	-9	-8	-83
ENSG00000177463:E020	ENSG00000177463	NR2C2	-1	-2	-4	-11	-2	-4	-16
ENSG00000187147:E032	ENSG00000187147	RNF220	-1	-3	-3	-14	-11	-5	-10

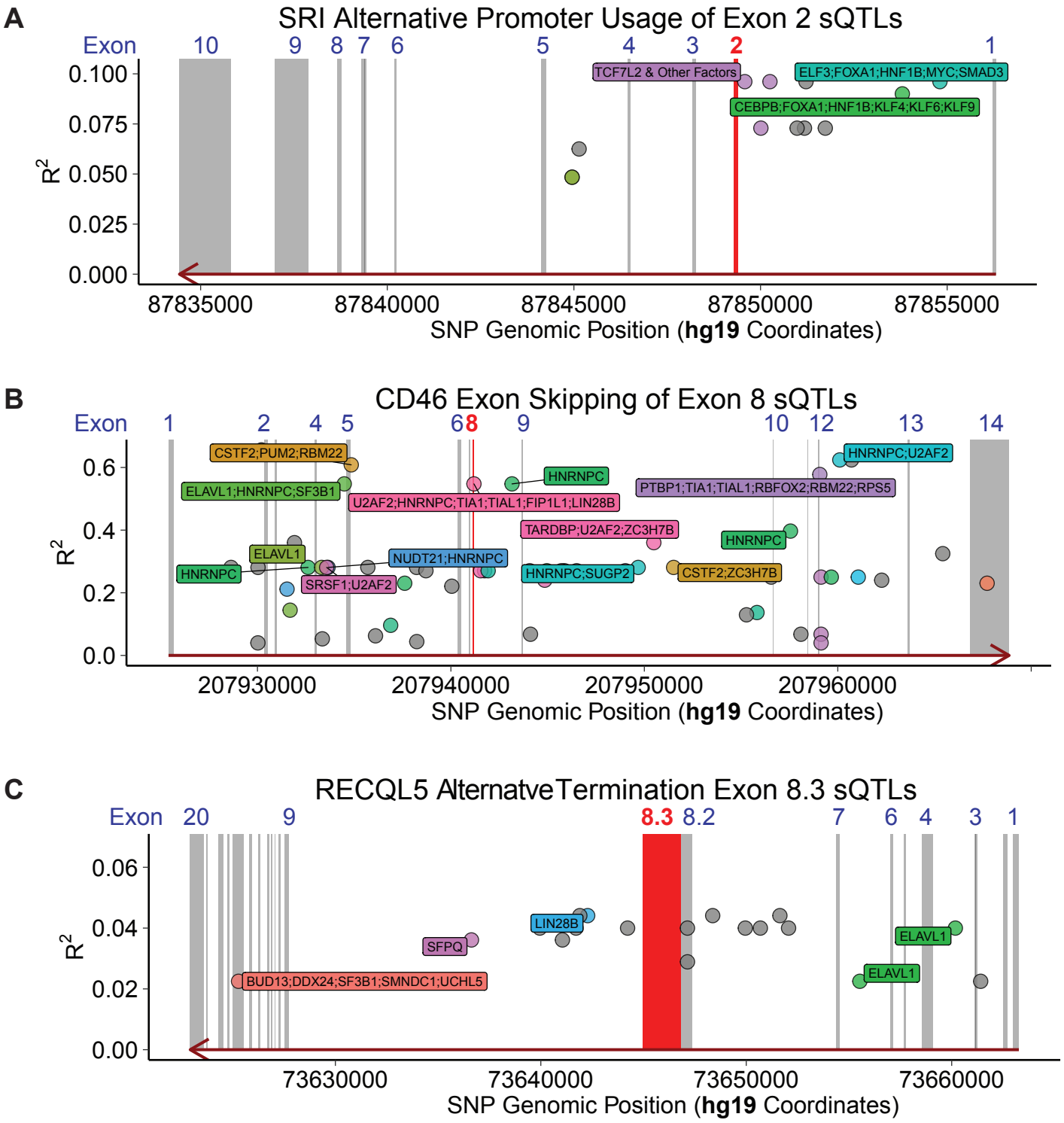
Supplementary Figure S8: A tabulation of the 35 isoform expression changes from 30 genes that were shared between the pancreatic and colorectal cancers from the RNA-seq datasets. This list was generated with the filters shown in main Figure 3A (|PSI| ≥ 10% & adjusted p-value ≤ 0.1). The magnitude of the differential variable exon usage (measured in ΔPSI) is shown in the table. The data that did not pass the aforementioned filters have been shaded out. More detailed information about these Wnt regulated isoform expression changes can be found in Supplementary Table 2.

SUPPLEMENTARY FIGURE S9



Supplementary Figure S9: Genes involved in Wnt regulated variable exon usage have important biological functions. Gene Ontology (GO) analysis of the genes that were found to be differentially spliced in at least one time point in either the HPAF-II orthotopic or colorectal PDX (ColoPDX) tumor models. Genes associated with two of the categories in the GO analysis are shown in the heatmaps below together with the magnitude of splicing change (ΔPSI) or the ETC-159 treated time points relative to the untreated controls. **(A)** Molecular function GO. **(B)** Cellular component GO. Hierarchical clustering was applied to each heatmap.

SUPPLEMENTARY FIGURE S10



Supplementary Figure S10: Genomic maps of the splicing Quantitative Trait Loci (sQTLs) and their associated exons for **(A)** the alternative promoter usage of *SRI* exon 2, **(B)** exon skipping of *CD46* exon 8 and **(C)** alternative termination of *RECQL5* exon 8.3. Genomic coordinates show the relative position of all the exons in the gene (highlighted in grey with the variable exon highlighted in red) and the sQTLs. The R2 values indicate the magnitude of the splicing (in terms of PSI values) that can be explained by the sQTL. The larger the R2 value, the stronger the association between the sQTL and the variable exon expression. sQTLs that overlap with known transcriptional regulator (in the case of *SRI* exon 2 **(A)**) and RNA-binding protein (for *CD46* exon 8 **(B)** and *RECQL5* exon 8.3 **(C)**) binding sites have been labelled.