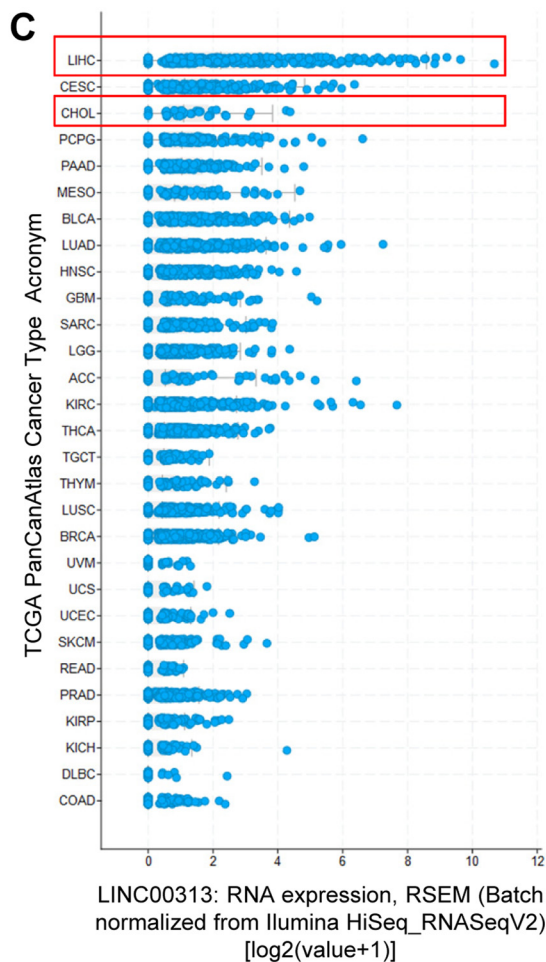
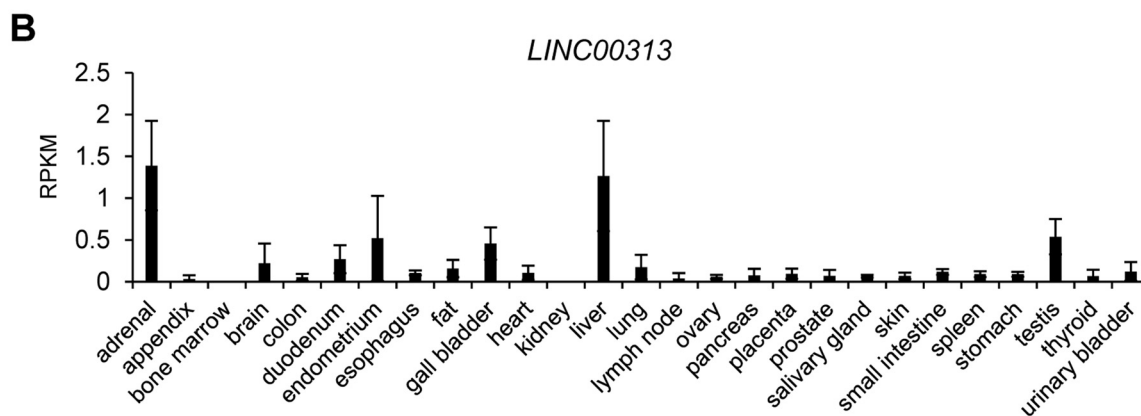
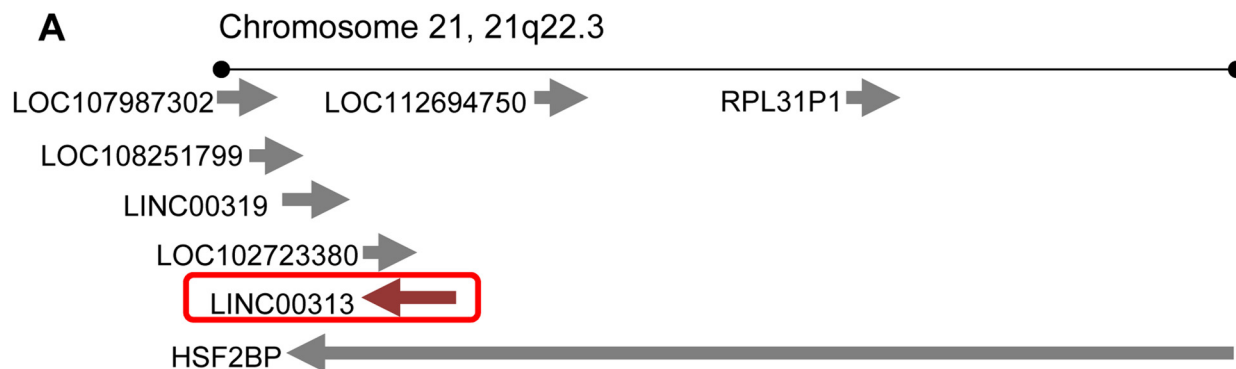


Expanded View Figures

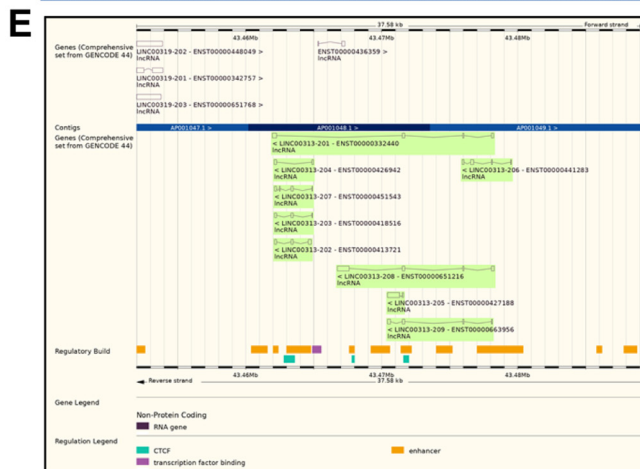
Figure EV1. Characterization of LINC00313.

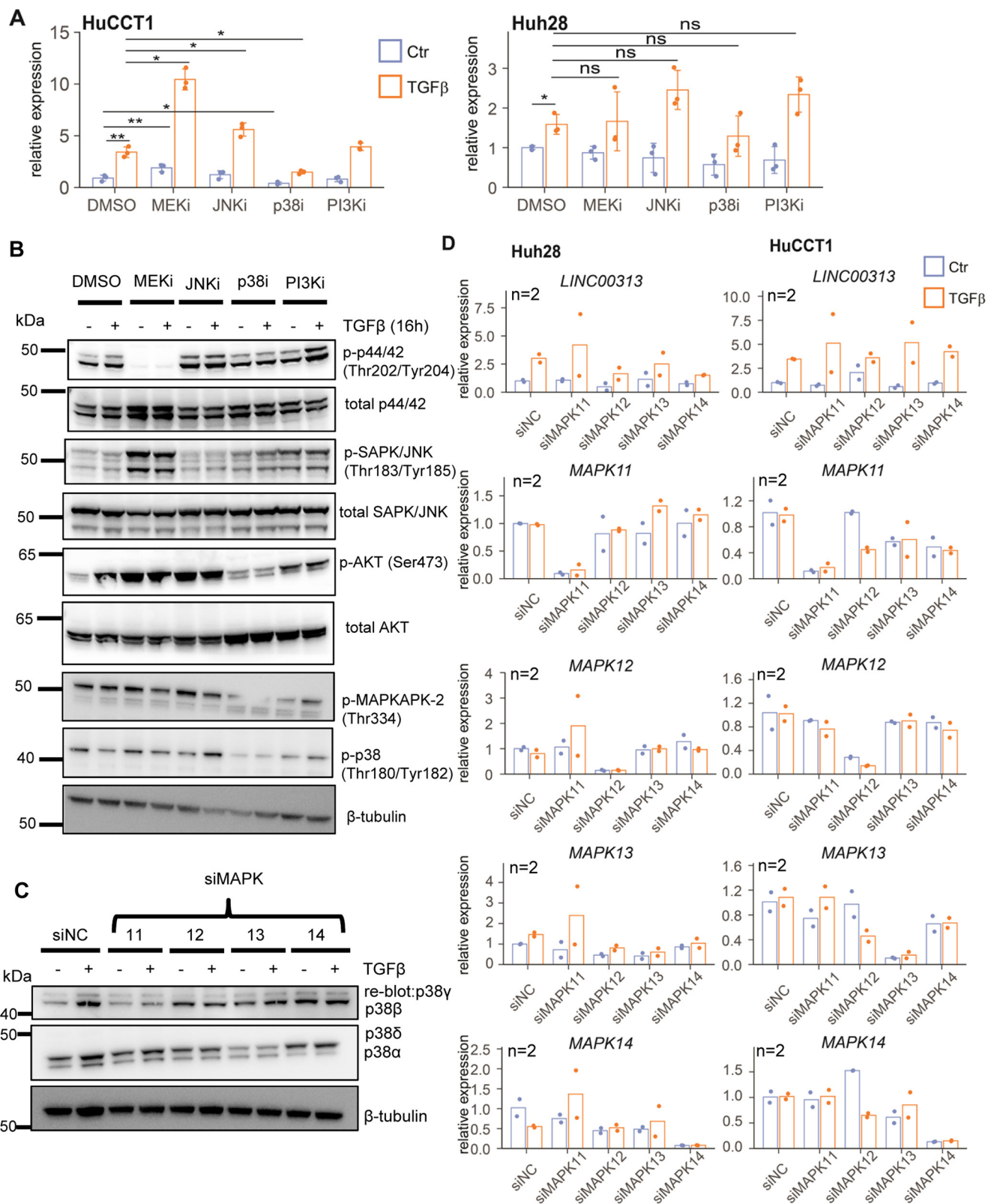
(A) Schematic representation of LINC00313 genomic locus on chromosome 21. (B) LINC00313 expression profile among 27 different human tissues from 95 individuals, as determined by RNA-seq (accession: PRJEB4337). (C) LINC00313 expression levels among 29 cancer types from the PanCancer Atlas of TCGA, sorted from the highest (top) to the lowest (bottom) expression. (D) ORF analysis of LINC00313 lncRNA sequence, using the Open Reading Frame Finder tool from NCBI. (E) Snapshot of the LINC00313 genomic locus from the Ensembl database showing the different splice variants. Data information: In panel (B) LINC00313 expression is presented in Reads Per Kilobase per Million mapped reads. In panel (C) LINC00313 expression is quantified with RSEM and displayed in logarithmic scale.



D

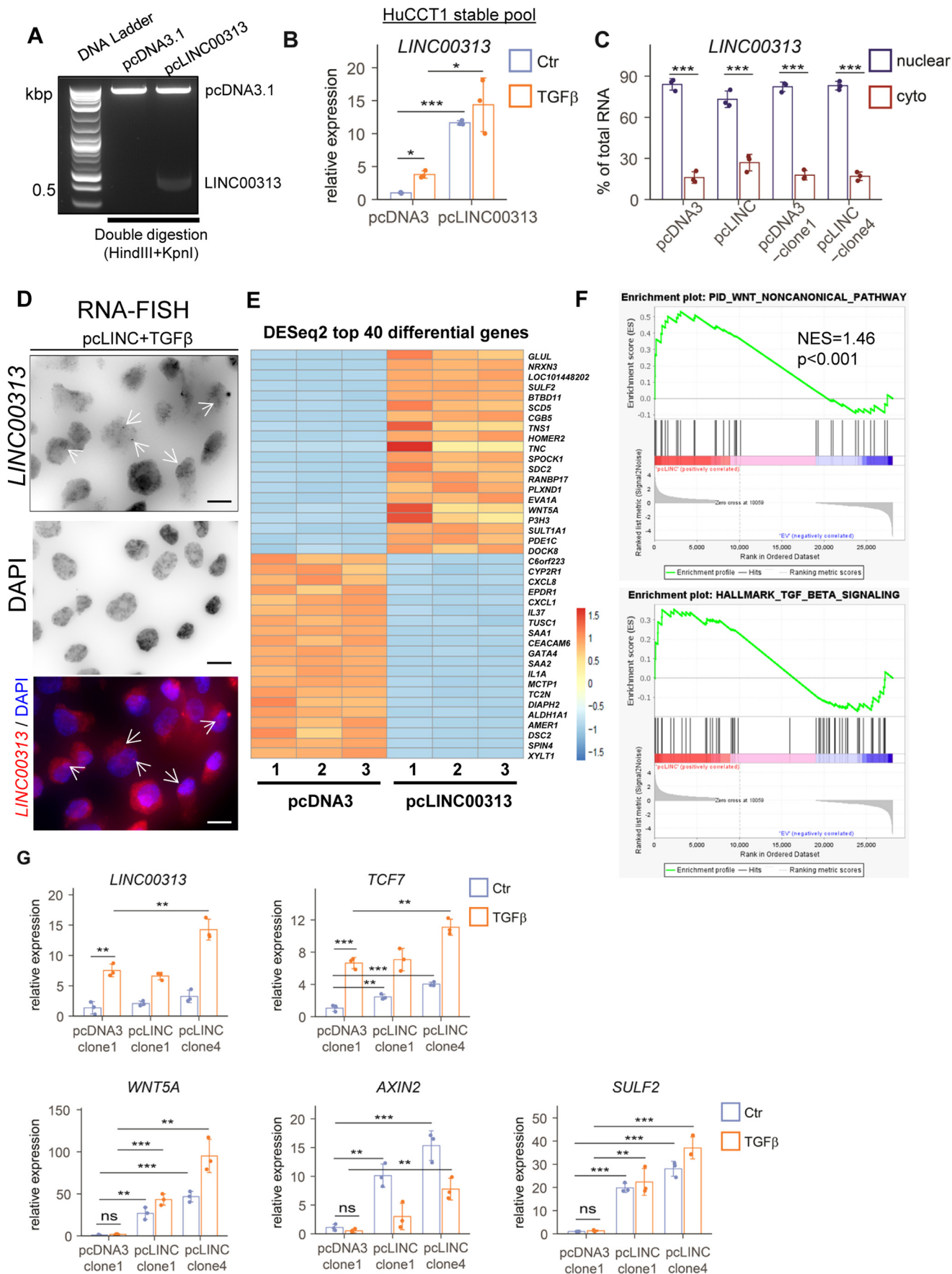
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ORF3	+	3	90	323	234 77
ORF6	-	2	566	390	177 58
ORF1	+	1	277	423	147 48
ORF4	+	3	369	488	120 39
ORF2	+	2	287	400	114 37
ORF7	-	3	91	>2	90 29





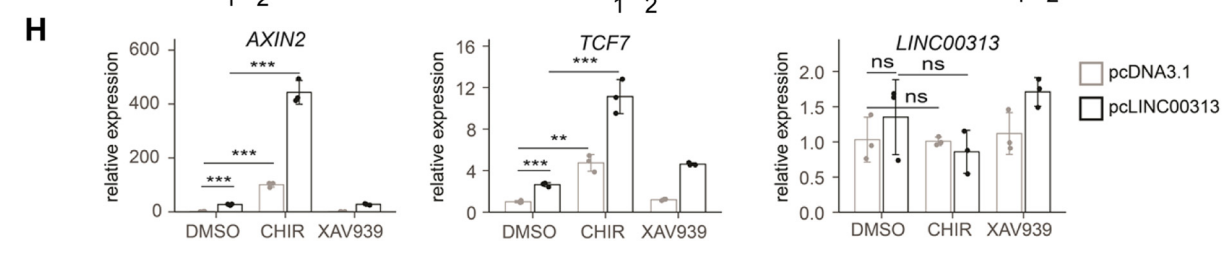
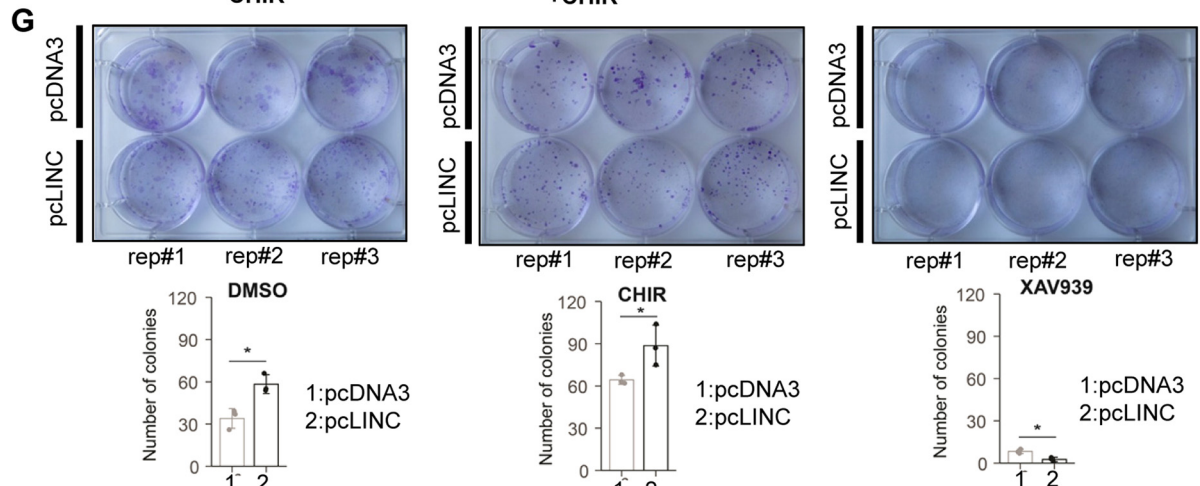
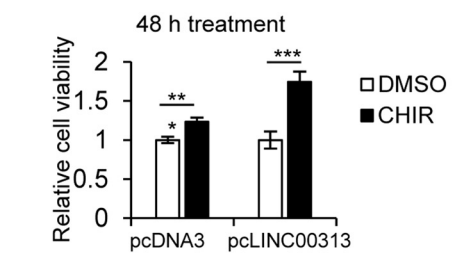
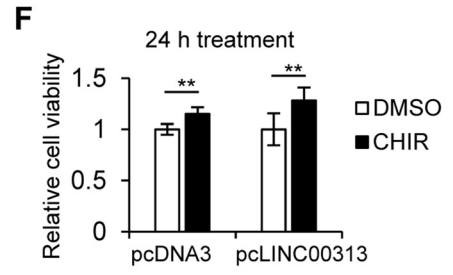
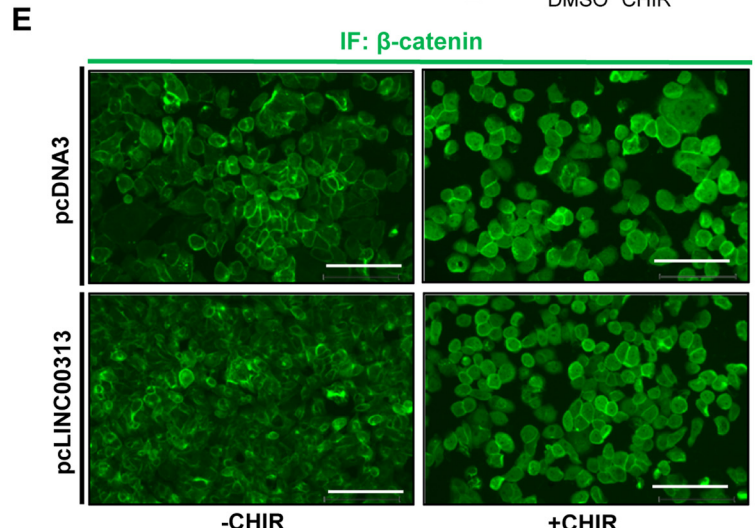
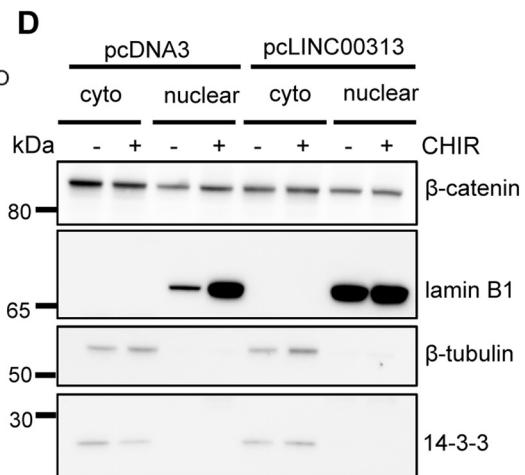
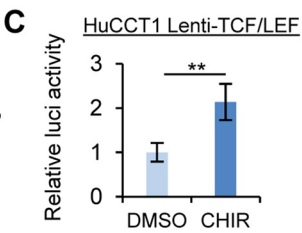
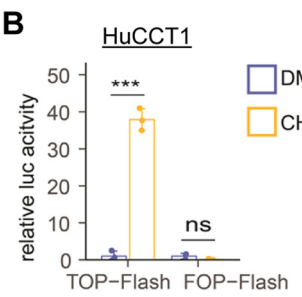
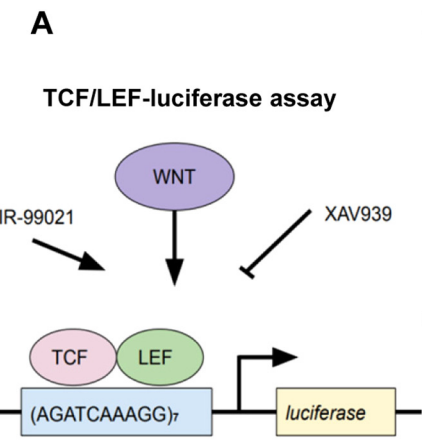
◀ Figure EV2. Effect of the non-canonical TGF β pathway on LINC00313 expression.

(A) *LINC00313* levels in HuCCT1 or Huh28 treated with MEK, p38, JNK or PI3K inhibitors, with or without TGF β 1 stimulation for 16 h ($n = 3$ biological replicates). (B) Immunoblotting for detection of phosphorylated p44/42 (Thr202/Tyr204), phosphorylated p-SAPK/JNK (Thr183/Tyr185), phosphorylated AKT (Ser473), phosphorylated p38 (Thr180/Tyr182), phosphorylated MAPKAPK-2 (Thr334), total p44/42, SAPK/JNK and AKT protein levels and β -tubulin (used as a loading control) in HuCCT1 cells treated with MEK, p38, JNK or PI3K inhibitors, with or without TGF β 1 stimulation for 16 h. DMSO was used as a vehicle treatment. (C) Immunoblotting for detection of the p38 isoforms p38 α , p38 β , p38 γ and p38 δ in HuCCT1 cells transiently silenced for *MAPK11* or *MAPK12* or *MAPK13* or *MAPK14* and treated or not with TGF β 1 for 16 h. β -tubulin was used as a loading control. (D) Real-time qPCR analysis of *LINC00313*, *MAPK11*, *MAPK12*, *MAPK13* and *MAPK14* expression in HuCCT1 and Huh28 cell lines transiently transfected with siRNAs targeting *MAPK11* or *MAPK12* or *MAPK13* or *MAPK14* and treated or not with TGF β 1 for 16 h. Data information: In Panel (A) data are presented as mean \pm SD ($n = 3$ biological replicates). In panel (D) data are presented as single data points ($n = 2$ biological replicates). * $P \leq 0.05$, ** $P \leq 0.01$, n.s.: not significant (Student's t -test). Source data are available online for this figure.



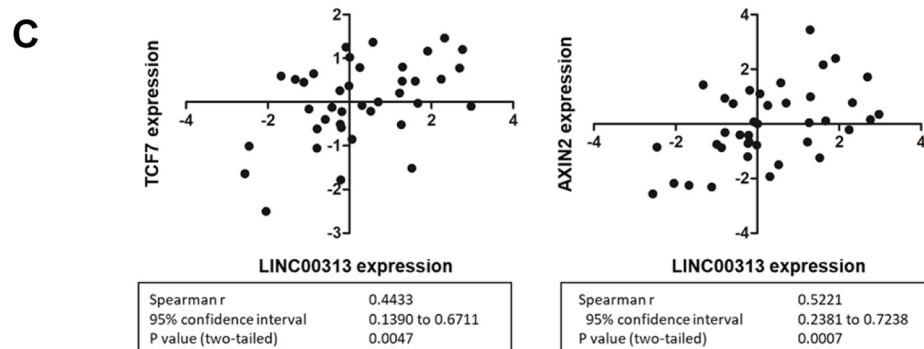
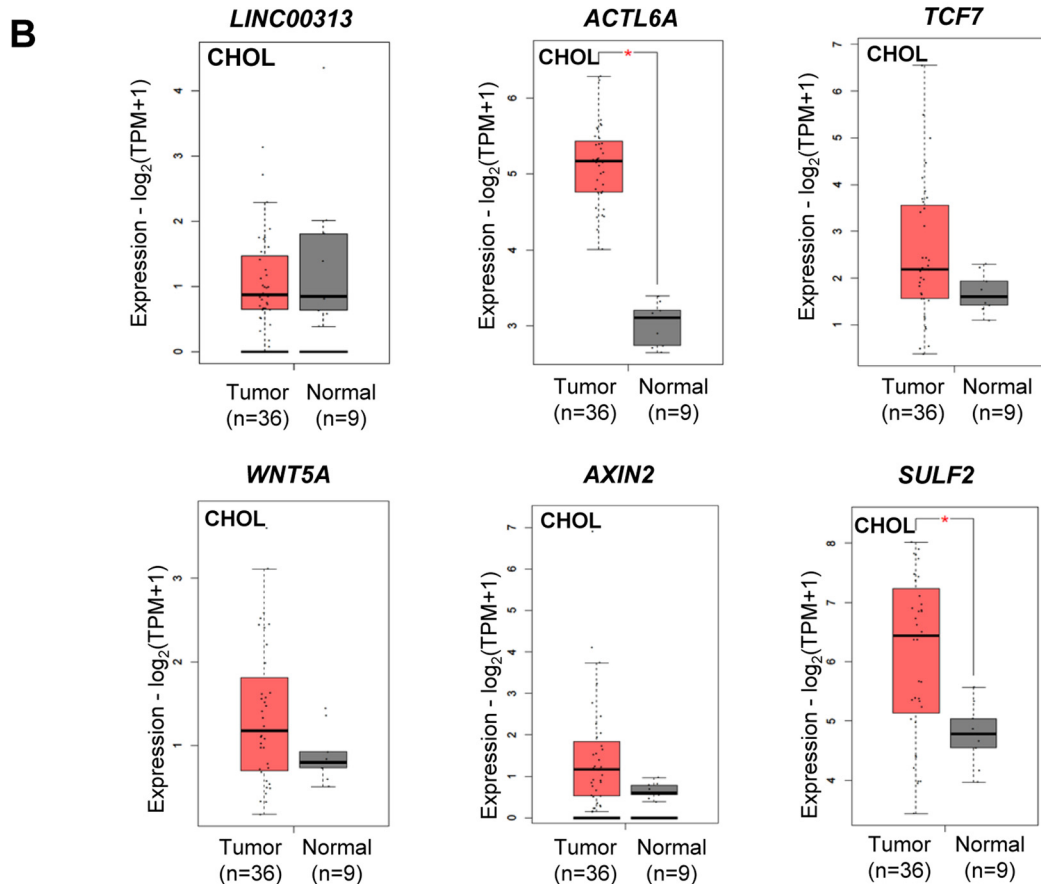
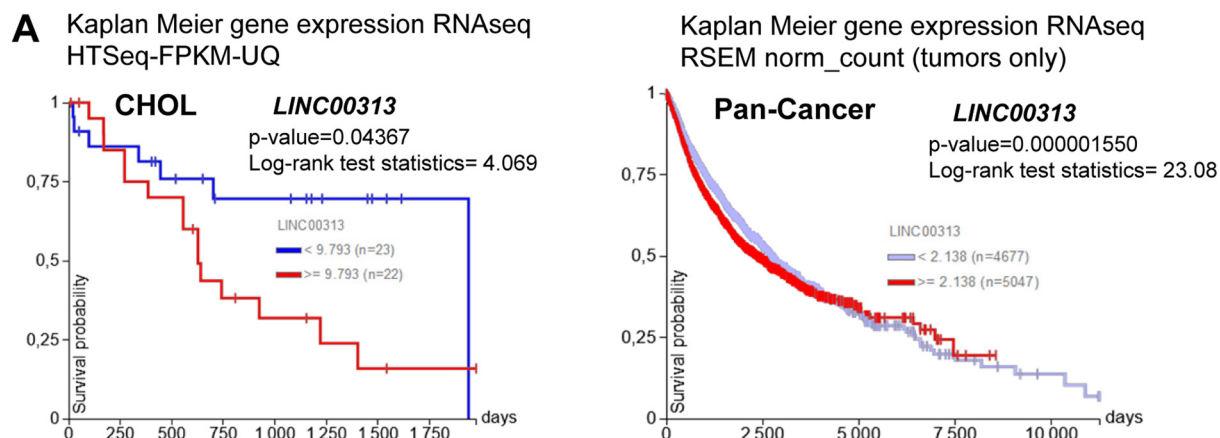
◀ Figure EV3. LINC00313 gain of function up-regulates Wnt-related genes.

(A) Efficient ligation of LINC00313 insert to pcDNA3.1 plasmid vector was verified by double digestion with restriction enzymes, followed by agarose gel electrophoresis. Molecular size (in kbp) marker ladder is shown in the first lane. (B) Real-time qPCR analysis of *LINC00313* expression in HuCCT1 cells stably over-expressing pcDNA3.1 or pcLINC00313 expression vectors stimulated or not with TGF β 1 for 16 h. (C) Subcellular fractionation followed by RT-qPCR analysis of LINC00313 RNA levels in nuclear and cytoplasmic fractions of HuCCT1 cells transiently over-expressing an empty vector (pcDNA3.1) or LINC00313 and HuCCT1 clones, stably over-expressing pcDNA3.1 or LINC00313. (D) smiFISH for LINC00313 RNA in HuCCT1 cells stably over-expressing LINC00313 and stimulated with TGF β for 16 h. DAPI was used to stain nuclei. Scale bars: 15 μ m. (E) Heatmap presenting the top 40 differentially regulated genes in HuCCT1 pcLINC00313 versus pcDNA3-expressing cells (shrunken log₂FC > 1, adjusted *p*-value < 0.1). Biological triplicates (1-3) were used per condition. (F) GSEA analysis of differentially expressed genes in pcLINC00313 versus pcDNA3 HuCCT1 cells. (G) Real-time qPCR analysis of *LINC00313*, *TCF7*, *WNT5A*, *AXIN2*, and *SULF2* expression in a monoclonal HuCCT1 cell line stably expressing pcDNA3.1 and in two monoclonal HuCCT1 cell lines stably expressing pcLINC00313, in the presence or not of TGF β 1 for 16 h. Data information: Data in panels (B), (C) and (G) are presented as mean \pm SD (*n* = 3 biological replicates). **P* \leq 0.05, ***P* \leq 0.01, ****P* \leq 0.001 (Student's *t*-test). In panel (F) the Kolmogorov-Smirnov test was used for statistical analysis of GSEA. Source data are available online for this figure.



◀ Figure EV4. *LINC00313* modulates chemically-induced Wnt/ β -catenin signalling and impacts physiological processes in CCA.

(A) Schematic representation of the TCF/LEF-luciferase reporter assay. Activators (Wnt ligands, CHIR) and inhibitors (XAV939) of TCF/LEF-dependent responses are also shown. (B) TCF/LEF-luciferase reporter assay in HuCCT1 cells transiently transfected with TOP-Flash or FOP-Flash luciferase expression vectors and treated with CHIR99021 or DMSO for 24 h. Cells were co-transfected with a Renilla luciferase expression vector for normalization of the firefly luciferase activity. (C) TCF/LEF-luciferase reporter assay in HuCCT1 cells stably expressing the pGreenFire 2.0 TCF/LEF reporter construct and treated with CHIR99021 or DMSO for 24 h. TCF/LEF-luciferase reporter assay in HuCCT1 cells stably expressing the pGreenFire 2.0 TCF/LEF reporter construct and treated with the indicated concentrations of TGF β 1 or BSA/HCl (Ctr) for 16 h. (D) Subcellular fractionation followed by immunoblotting for β -catenin, lamin B1 (nuclear marker), β -tubulin and 14-3-3 (cytoplasmic markers) using nuclear and cytoplasmic fractions of HuCCT1 cells stably over-expressing an empty vector (pcDNA3.1) or LINC00313 and treated with CHIR99021 or DMSO for 24 h. (E) Immunofluorescence to detect β -catenin subcellular localization in control or LINC00313 over-expressing HuCCT1 cells, treated or not with CHIR99021 or DMSO for 24 h. Scale bars: 150 μ m. (F) Cell viability assays in control or LINC00313 over-expressing HuCCT1 cells treated with CHIR99021 or DMSO for 24 h. (G) Colony formation assay in control or LINC00313 over-expressing HuCCT1 cells, treated with CHIR99021 or XAV939 or DMSO for 24 h. Quantification of the number of colonies for each condition is also shown. (H) Real-time qPCR analysis of *AXIN2*, *TCF7* and *LINC00313* expression in control or LINC00313 overexpressing HuCCT1 cells, treated with CHIR99021, XAV939, or DMSO for 24 h. Data information: In panel (B) data are presented as mean \pm SD ($n = 3$ biological replicates). In panel (F) data are presented as mean \pm SD ($n = 6$ biological replicates). ** $P \leq 0.01$, *** $P \leq 0.001$ (Student's t -test). In panels (G) and (H) data are presented as mean \pm SD ($n = 3$ biological replicates). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ (Student's t -test). Source data are available online for this figure.



◀ Figure EV5. Clinical relevance of LINC00313 in CCA.

(A) Kaplan Meier curves depicting overall survival in CHOL patients and Pan-Cancer patients grouped based on *LINC00313* median expression, using the Xena Functional Genomics Explorer from the UCSC. (B) Boxplots of *LINC00313*, *ACTL6A*, *TCF7*, *WNT5A*, *AXIN2* and *SULF2* transcripts per million (TPMs) in cholangiocarcinoma (CHOL) tumours ($n = 36$) and their paired normal tissues ($n = 9$), using GEPIA2. * $p < 0.01$. (C) Expression correlation of *LINC00313* with *TCF7* and *AXIN2* in a cohort of 39 iCCA patients $p < 0.05$. Data information: In panel (A), log-rank test was used to compare the different Kaplan–Meier curves. In panel (B), transcript expression is shown in logarithmic scale and the method for differential analysis is one-way ANOVA, using disease state (tumour vs normal) as variable for calculating differential expression. The \log_2FC cut-off equals 1 and the p -value cut-off equals 0.01. In panel (C) a two-tailed Student's t -test was used to calculate p -values.