**ISCI, Volume *4***

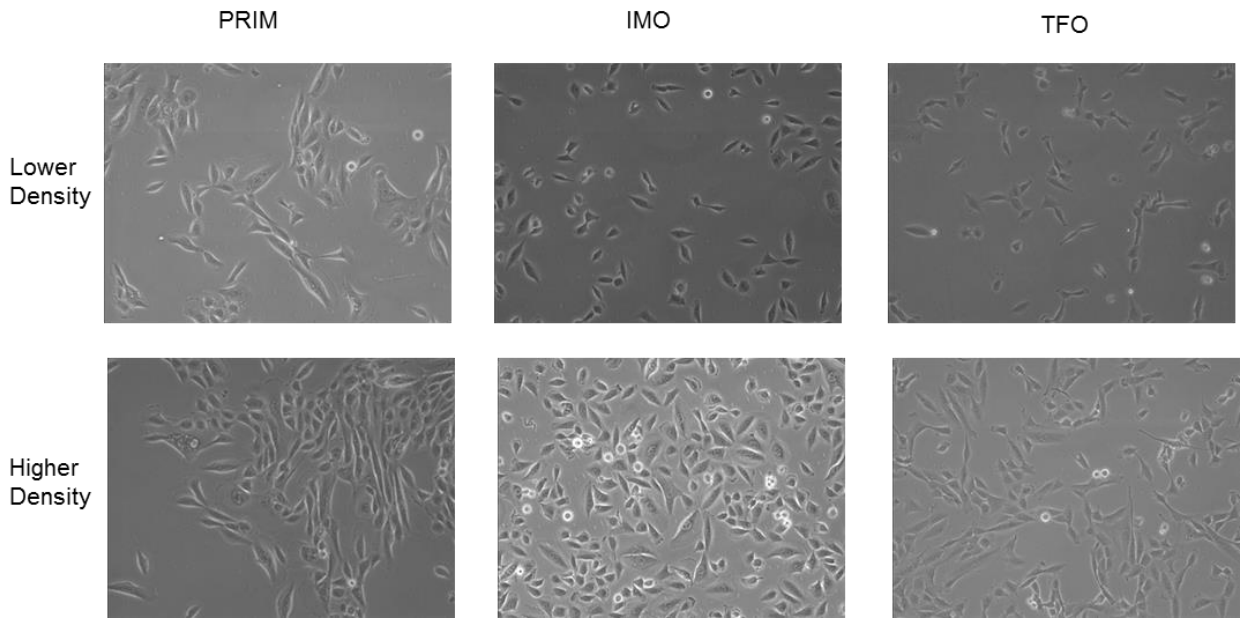
## Supplemental Information

# Ras Post-transcriptionally Enhances a Pre-malignantly Primed EMT to Promote Invasion

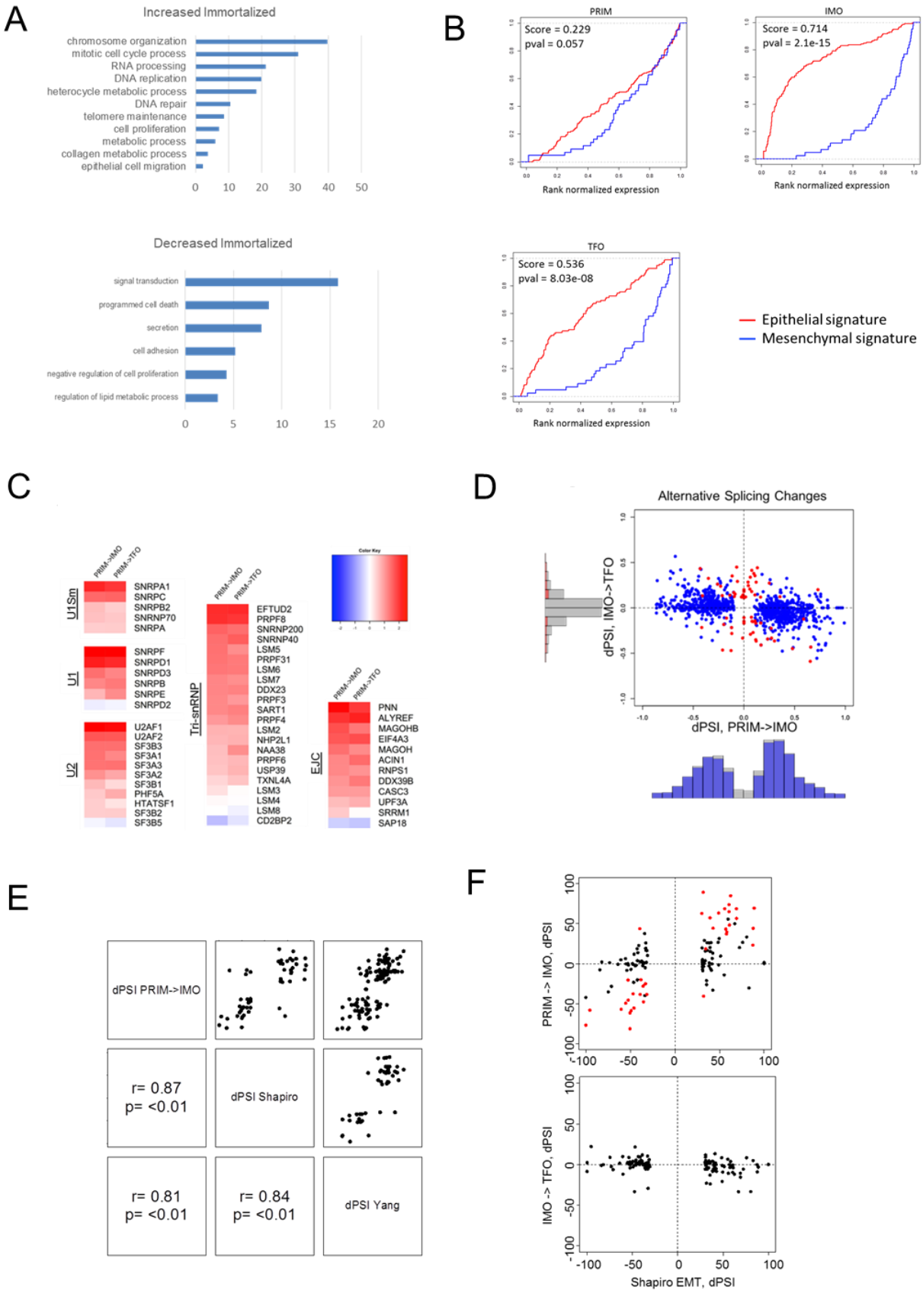
**Laura S. Bisogno, Matthew B. Friedersdorf, and Jack D. Keene**

**Supplemental Information:**

**Supplemental Figures and Legends:**




**Supplemental Figure 1:**
**PRIM cells are epithelial, while IMO and TFO cells are morphologically more mesenchymal. Related to Figure 1.** Phase contrast micrographs of Primary (PRIM), Immortalized (IMO), and Transformed (TFO) HMECs at low confluency (top) and high confluency (bottom).


A
Increased Immortalized
chromosome organization
mitotic cell cycle process
RNA processing
DNA replication
heterocycle metabolic process
DNA repair
telomere maintenance
cell proliferation
metabolic process
collagen metabolic process
epithelial cell migration
0 10 20 30 40 50
Decreased Immortalized
signal transduction
programmed cell death
secretion
cell adhesion
negative regulation of cell proliferation
regulation of lipid metabolic process
0 5 10 15 20
B
PRIM
Score = 0.229
pval = 0.057
Rank normalized expression
IMO
Score = 0.714
pval = 2.1e-15
Rank normalized expression
TFO
Score = 0.536
pval = 8.03e-08
Rank normalized expression
Epithelial signature
Mesenchymal signature
C
PRIM->IMO
PRIM->TFO
U1Sm
SNRPA1
SNRPC
SNRPB2
SNRNP70
SNRPA
U1
SNRPF
SNRPD1
SNRPD3
SNRPB
SNRPE
SNRPD2
U2
U2AF1
U2AF2
SF3B3
SF3A1
SF3A3
SF3A2
SF3B1
PHF5A
HTATSF1
SF3B2
SF3B5
Tri-snRNP
EFTUD2
PRPF8
SNRNP200
SNRNP40
LSM5
PRPF31
LSM6
LSM7
DDX23
PRPF3
SART1
PRPF4
LSM2
NHP2L1
NAA38
PRPF6
USP39
TXNL4A
LSM3
LSM4
LSM8
CD2BP2
EJC
PNN
ALYREF
MAGOHB
EIF4A3
MAGOH
ACIN1
RNPS1
DDX39B
CASC3
UPF3A
SRRM1
SAP18
D
Alternative Splicing Changes
dPSI, IMO->TFO
dPSI, PRIM->IMO
E
dPSI PRIM->IMO
r= 0.87
p= <0.01
dPSI Shapiro
r= 0.81
p= <0.01
r= 0.84
p= <0.01
dPSI Yang
F
PRIM -> IMO, dPSI
IMO -> TFO, dPSI
Shapiro EMT, dPSI

**Supplemental Figure 2:**
**IMO cells have a mesenchymal mRNA expression and alternative splicing signatures that are maintained in TFO cells. Related to Figure 2.**

A) Enriched GO categories for genes increasing (upper group) or decreasing (lower group) during primary cell immortalization.

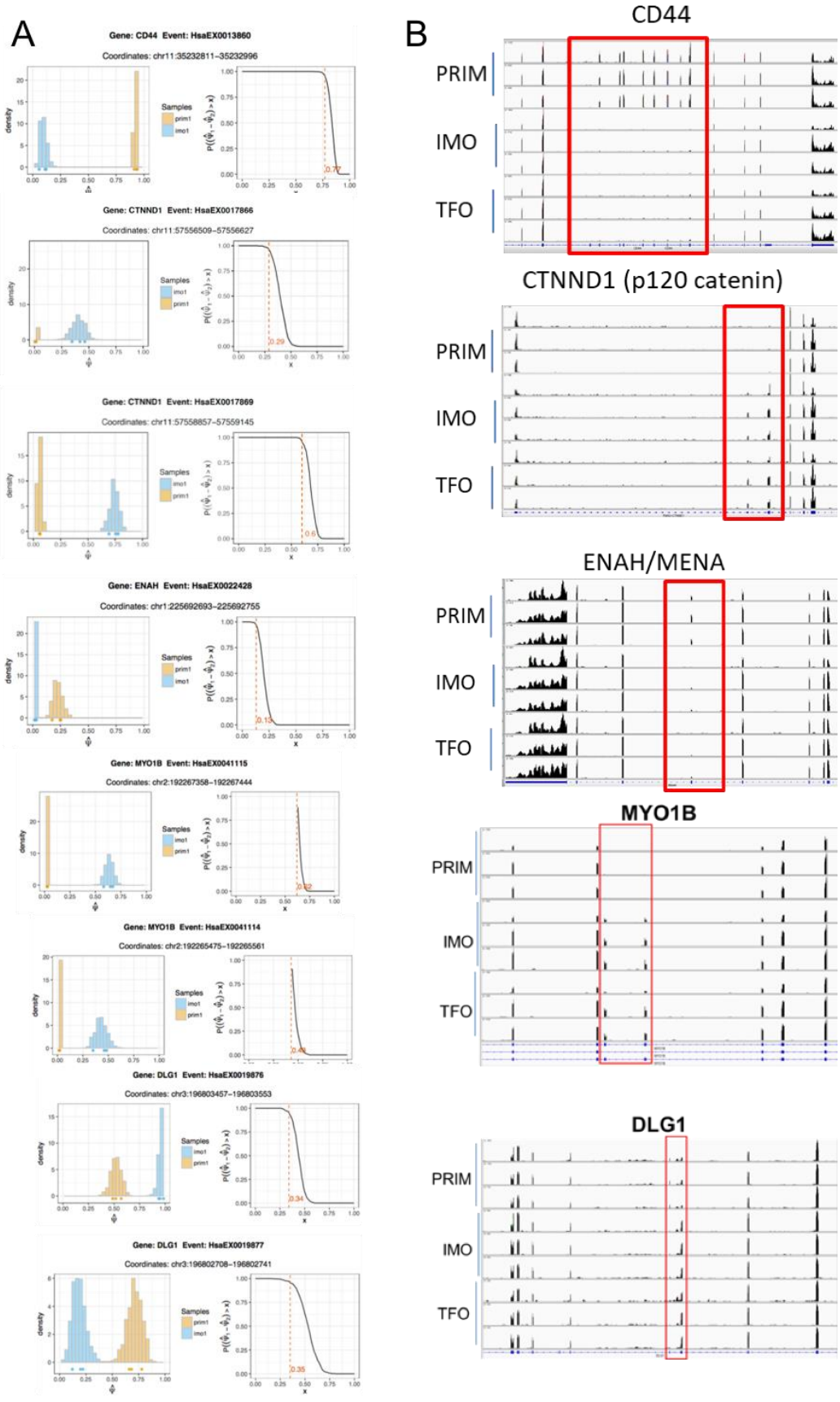
B) Previously reported epithelial and mesenchymal gene expression signatures (Tan et al. 2014) were used to generate empirical distribution functions for PRIM, IMO and TFO cells. The X-axis shows ranked normalized expression, with "1" being the most abundant and "0" being the least abundant. EMT signature scores for PRIM, IMO and TFO HMECs were calculated using a two-sample Kolmogorov-Smirnov test. The EMT signature score is reported as a value between -1 and +1, with more positive scores indicating a more mesenchymal state.

C) Expression of mRNA encoding core splicing components increase significantly during immortalization. Heatmap depicts changes from PRIM to IMO and from PRIM to TFO.

D) Significant changes in alternative splicing are observed during immortalization. dPSI= change in percent spliced in. Blue is significant reproducible alternative splicing events from PRIM to IMO and red is significant reproducible alternative splicing events from IMO to TFO.

E) Pair-wise comparison of alternative splicing changes induced during immortalization to previously reported EMT-signatures (Shapiro et al. 2011; Yang et al. 2016). R-values indicate correlation coefficients for each comparison. Top middle box is the comparison between immortalization and Shapiro et al. 2011, R=0.87. Top right box is the comparison between immortalization and Yang et al. 2016, R=0.81. Middle right box is the comparison between Shapiro et al. 2011 and Yang et al. 2016, R=0.84.

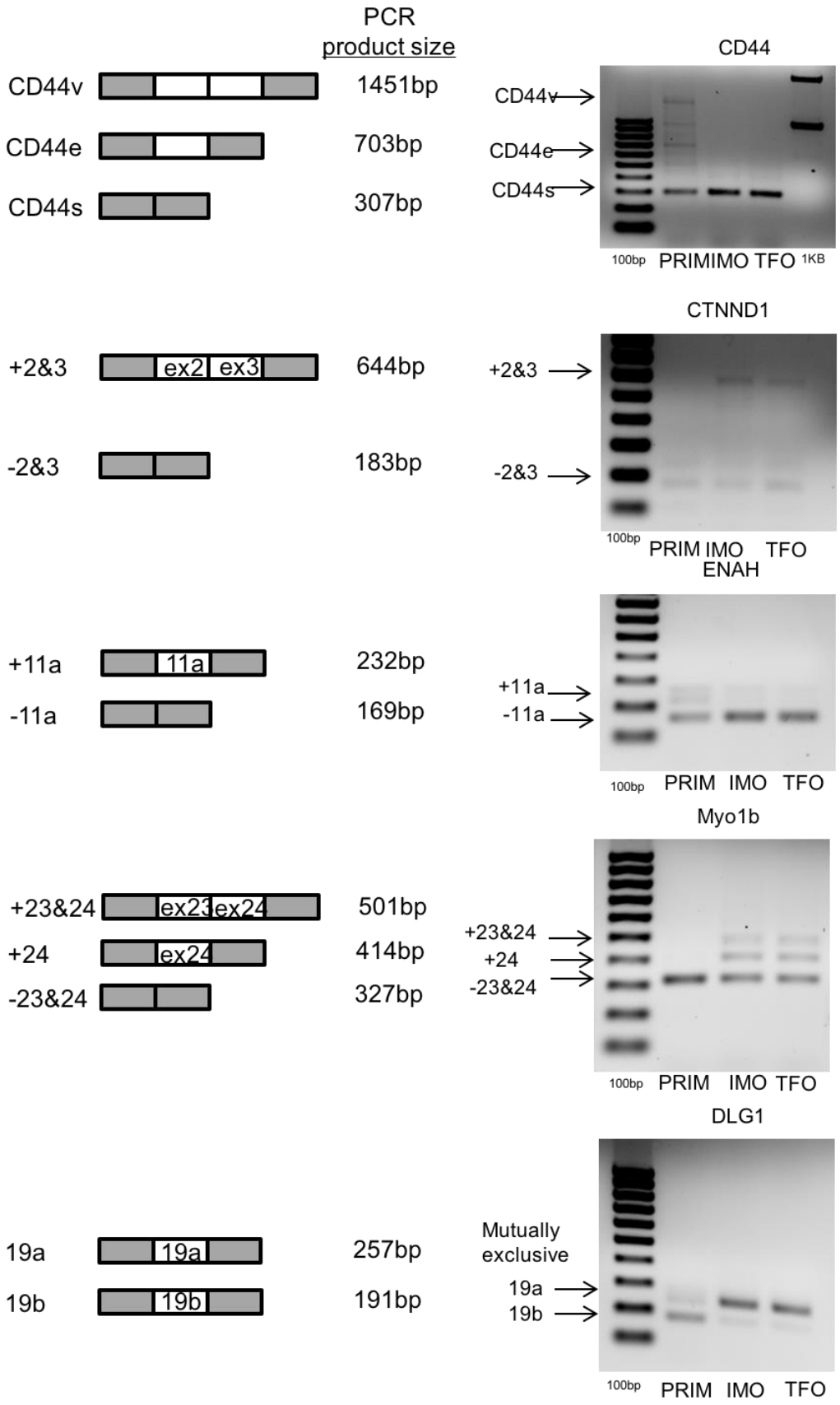
F) Comparison of alternative splicing events reported by Shapiro et al. to immortalization (top) and transformation (bottom).


A
Gene: CD44 Event: HsaEX0013860
Coordinates: chr11:35232811−35232996
Gene: CTNND1 Event: HsaEX0017866
Coordinates: chr11:57556509−57556627
Gene: CTNND1 Event: HsaEX0017869
Coordinates: chr11:57558857−57559145
Gene: ENAH Event: HsaEX0022428
Coordinates: chr1:225692693−225692755
Gene: MYO1B Event: HsaEX0041115
Coordinates: chr2:192267358−192267444
Gene: MYO1B Event: HsaEX0041114
Coordinates: chr2:192265475−192265561
Gene: DLG1 Event: HsaEX0019876
Coordinates: chr3:196803457−196803553
Gene: DLG1 Event: HsaEX0019877
Coordinates: chr3:196802708−196802741
Samples
prim1
imo1
density
B
CD44
CTNND1 (p120 catenin)
ENAH/MENA
MYO1B
DLG1
PRIM
IMO
TFO

**Supplemental Figure 3:**
**Visualization and confirmation of well-described (CD44, CTNND1 and ENAH) and novel (Myo1b and DLG1) alternative splicing events during EMT. Related to Figure 2.**

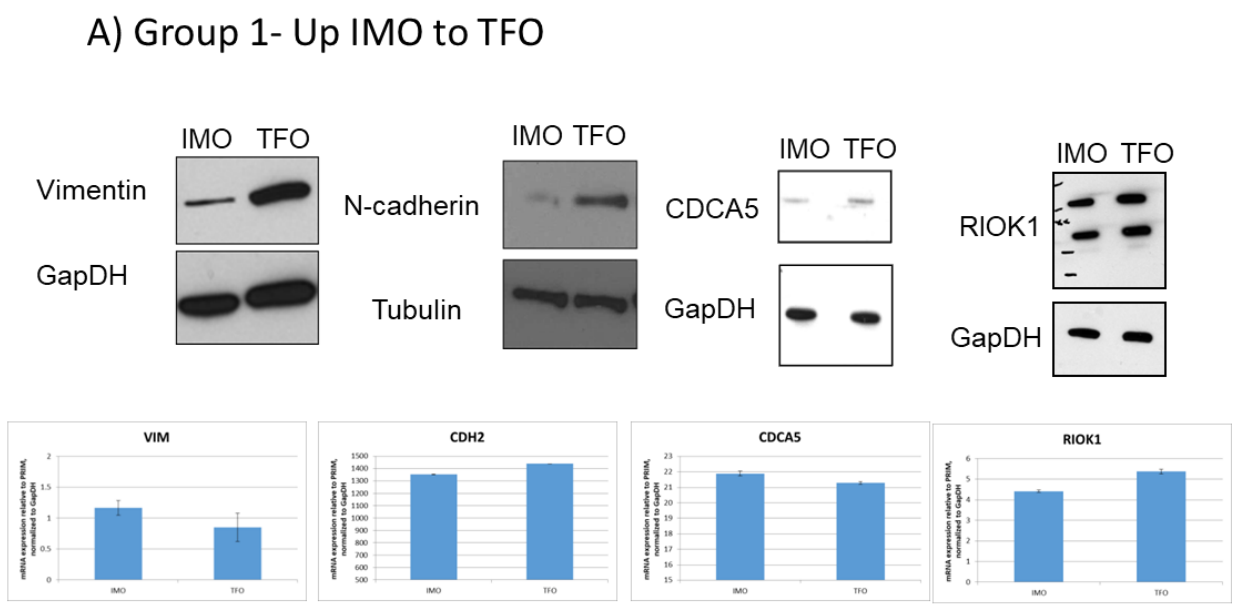
A) Visualizations of VAST analysis. The plots in the left panels show the two joint posterior distributions over psi. Each replicate is plotted as dots below the histogram. The right panels show the probability (y-axis) of delta psi being greater than a given value (x-axis). The red line is the 95% confidence interval.

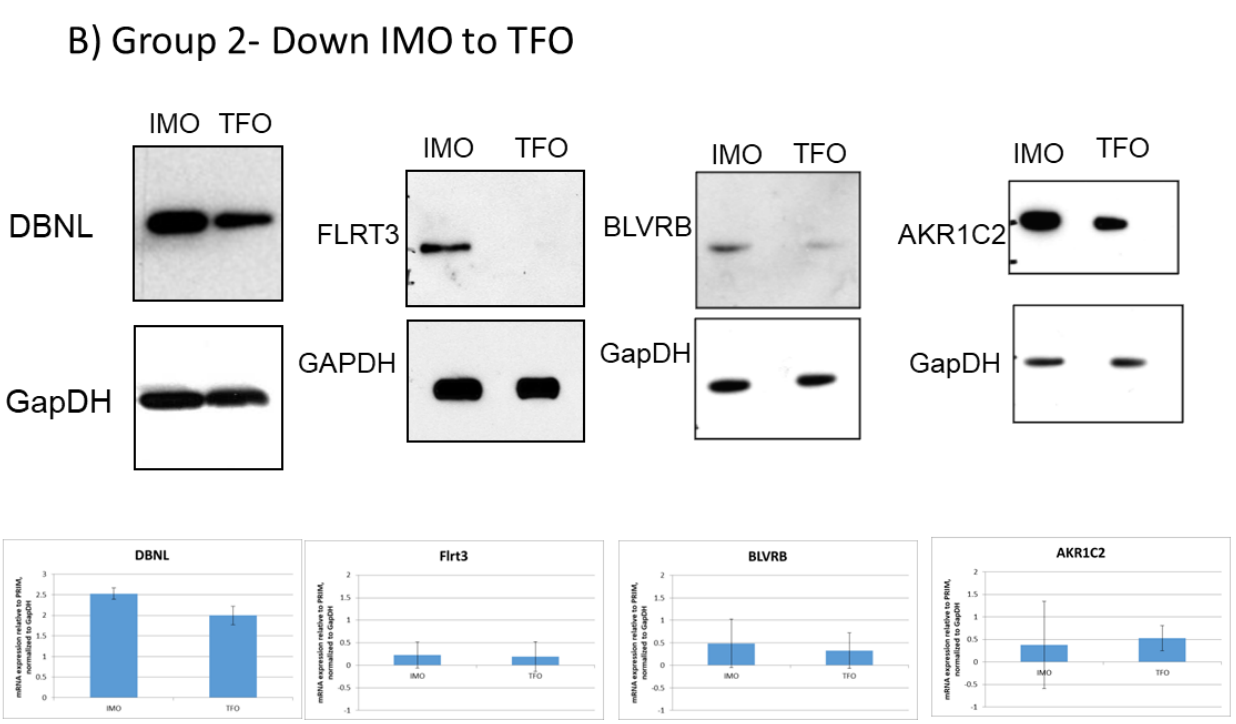
B) IGV screen shots of alternative splicing events, indicated by red boxes.


PCR
product size
CD44v
1451bp
CD44e
703bp
CD44s
307bp
CD44
CD44v
CD44e
CD44s
100bp PRIMIMO TFO 1KB
+2&3
ex2 ex3
644bp
-2&3
183bp
CTNND1
+2&3
-2&3
100bp PRIM IMO TFO
ENAH
+11a
11a
232bp
-11a
169bp
+11a
-11a
100bp PRIM IMO TFO
Myo1b
+23&24
ex23ex24
501bp
+24
ex24
414bp
-23&24
327bp
+23&24
+24
-23&24
100bp PRIM IMO TFO
DLG1
19a
19a
257bp
19b
19b
191bp
Mutually
exclusive
19a
19b
100bp PRIM IMO TFO

**Supplemental Figure 4:**
**PCR confirmation of alternative splicing events. Related to Figure 2.**

Splice products depiction, name and expected size. PCR validation of alternative splicing events in PRIM, IMO, and TFO cells.




**Supplemental Figure 5:**
**Ras-transformation alters expression of proteins involved in EMT-related processes without altering corresponding mRNA levels. Related to Figures 3 and 4.**

Western blot validation of candidate Ras-regulated proteins identified by global proteomics data and qRT-PCR validation of RNA-seq data for mRNAs encoding Ras-regulated proteins that A) increase or B) decrease in protein but not mRNA abundance during oncogenic-Ras transformation.

**Transparent Methods:**

**Cell Culture**

HMECs were obtained from Lonza at p9 and maintained in Mammary Epithelial Growth Medium (MEGM; Lonza, Walkersville, MD, USA). IMO and TFO HMECs were maintained in MEGM+10% fetal bovine serum (FBS). Cells were passaged at 50-70% confluency using a Reagent Pack (Lonza) according to the manufacturer's subculturing protocol. All HMECs were authenticated by analysis at the Duke University DNA Analysis Facility. Cells were immortalized and transformed as previously described(Bisogno and Keene, 2017) (Bisogno and Keene, 2017); also see Supplemental Experimental Procedures. 293T/17 packaging cells were obtained from the Duke University Cell Culture Facility and maintained in Dulbecco's Modified Eagle's Medium (ThermoFisher Scientific, Grand Island, NY, USA) +10% FBS. All cells tested mycoplasma negative.

**Wound healing scratch assay**

Cells were grown in a single monolayer in 6-well plates. Cells were serum starved overnight and during experiment to control for proliferation. A single scratch was made with a p200 pipette tip and the scratch was photographed in 3 distinct places. After 24 hours the scratch was imaged in the same 3 locations, and percent wound closure was quantified using ImageJ's MRI wound healing tool. Data is reported as the mean and standard deviation of 4 replicates.

**Matrigel Invasion Assay**

Matrigel membrane matrix (Corning, Corning, NY, USA), was thawed on ice overnight and diluted to 300ug/ml in cold coating buffer (0.01M Tris pH 8, 0.7% NaCl, filtered). 100ul of Matrigel was added to 6.5mm Transwell with 8.0um Polycarbonate membrane inserts (Corning), and allowed to solidify at 37C for 2-4 hours. Cells were harvested with Trypsin/EDTA, washed 3 times with serum free media, and resuspended in serum free media at ~50,000 cells/ml. 500ul of cell suspension was added to the upper chamber. 750ul of media with serum was added to the lower chamber. No Matrigel control inserts were used in every experiment. After 16-18 hours incubation, cells that did not invade were scraped off the top chamber with a cotton swab. Cells were fixed in methanol and stained with 1% Toluidine Blue or 0.2% Crystal Violet. 4 distinct fields were photographed. Data is reported as the mean and standard deviation of 3 replicates, quantified as percent that migrated through Matrigel vs. through the membrane without Matrigel.

**RNA-sequencing**

RNA samples were sequenced on the Illumina Hi-Seq 2000/2500 platform (one replicate; 125bp PE) and the Illumina Hi-Seq 4000 platform (two replicates; 150bp PE) (See Supplemental Experimental Procedures). We obtained approximately 60-80 million reads per library. Reads were mapped to the human genome (hg19) using TopHat2(Kim et al., 2013), and significant changes in gene expression patterns were determined using Cufflinks/Cuffdiff(Trapnell et al., 2010). Gene ontology was analyzed using GOrilla (Eden et al., 2007; Eden et al., 2009). Alternative splicing was analyzed using Vast-tools (Irimia et al., 2014). RNA abundance for genes of interest were validated using qRT-PCR, and alternative splicing events of interest were validated with PCR and 1% agarose gel (See Supplemental Experimental Procedures).

**UPLC-MS/MS**

Samples were analyzed using a nanoACQUITY UPLC system (Waters Corporation, Milford, MA) coupled to a Q Exactive HF Orbitrap high resolution accurate mass tandem mass spectrometer (ThermoFisher Scientific, Bremen, Germany) via a nanoelectrospray ionization source (See Supplemental Experimental Procedures). Individual proteins of interest were validated by Western blotting (See Supplemental Experimental Procedures).

**ADD1 shRNA**

TRC1 lentiviral plasmids targeting ADD1 or a non-target negative control were obtained from Duke University's Functional Genomics shared resource. To control for off-target effects, 3 individual shRNAs and a non-targeting shRNA were used (See Supplemental Experimental Methods).

**Establishing a primary cell-derived system of tumorigenesis**

293T/17 cells were co-transfected with p0467/pcl-10A (gift of Dr. Chris Counter) and individual pBabe plasmids containing transgenes of interest (Table) using FuGENE-6 (Roche, Basel, Switzerland). 24 hours later, the transfection was repeated. 8 hours after the second transfection, the media was replaced with fresh MEGM, and cells were incubated in this new media for 48 hours. Amphotropic retrovirus-containing media was harvested from the cells and filtered through a sterile 0.45µm Acrodisc with an HT tuffryn membrane (VWR, Radnor, PA). All media was snap-frozen in liquid nitrogen and stored at -80 C. Fresh MEGM was added to the 293T/17 cells, and 12 hours later media was harvested, filtered and snap frozen as done previously. Media containing the pBabe-hTERT amphotropic retroviruses was thawed at 37C. Polybrene (hexadimethrine bromide, Sigma-Aldrich, St. Louis, MO) was added to a concentration of 4µg/ml, and the media was added to primary HMECs. 12 hours later, the procedure was repeated with the second pBabe-hTERT amphotropic retrovirus containing media. This procedure was repeated every 12 hours until the HMECs were transduced with each plasmid listed in Table 1. Cells without H-Ras were saved as the immortalized (IMO) cell line. Cells that were Ras-transformed are referred to as the transformed (TFO) cell line. Cells were selected sequentially with the following antibiotics, with a 5 day recovery between drug selections: Hygromycin: 80µg/ml for 7 days; G418: 250µg/ml for 10 days; Puromycin: 0.5µg/ml for 5 days; Zeocin: 800µg/ml for 8 days; Blasticidin: 4.5µg/ml for 7 days. RAS-transformed cells were sorted by flow cytometry for YFP expression. Transgene expression was tested with qRT-PCR, UPLC/MS-MS, and anchorage-independent growth was assessed with a soft agar assay.

| Transgene | Selection Marker | Source |
|---|---|---|
| hTERT | Hygromycin | addgene plasmid #1773, gift of Dr. Bob Weinberg (Counter et al., 1998) |
| $p53^{DD}$ | G418 | gift of Dr. Chris Counter (Hahn et al., 2002) |
| Cyclin D1 | Puromycin | addgene plasmid #9050, gift of Dr. William Hahn |
| $CDK4^{R24C}$ | Zeocin | gift of Dr. Chris Counter (Hahn et al., 2002) |
| $C\text{-}myc^{T58A}$ | Blasticidin | gift of Dr. Chris Counter (Yeh et al., 2004) |
| $H\text{-}RAS^{G12V}$ | YFP | gift of Dr. Chris Counter (Hahn et al., 1999) |
| Control | GFP | addgene plasmid #10668, gift of Dr. William Hahn |

**Table S2: Plasmids used to generate a genetically-defined system of tumorigenesis. Realated to Figure 1.**

**RNA-sequencing**

Total RNA was obtained from cells using the TRIsure reagent (Bioline, Luckenwalde Germany) according to the manufacturer's protocol. RNA quantity was analyzed using a NanoDrop spectrophotometer, and ribosomal RNA was depleted using the Ribozero Gold kit (Epicentre, Madison, WI, USA). 50ng of rRNA depleted RNA was used as input for sequencing libraries, which were made using the ScriptSeq v2 RNA-seq library preparation kit (Epicentre) according to the manufacturer's instructions. 16 amplification cycles were used in the final PCR amplification step. Libraries were checked with a BioAnalyzer, and sequenced on the Illumina Hi-Seq 2000/2500 platform (one replicate; 125bp PE) and the Illumina Hi-Seq 4000 platform (two replicates; 150bp PE) at Duke University's Center for Genomic and Computational Biology.

**Quantitative Real-time PCR**

Total RNA was isolated from cells using TriSure (Bioline, Luckenwalde Germany) and reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA), both according to the manufacturer's recommendations. Quantitative real-time PCR was performed using the Roche Lightcycler with Sybr green detection (Invitrogen, Carlsbad, CA). Amplification of a single product was confirmed by melting curve analysis, and the ΔΔCt analysis method was used. Data are reported as mean and standard deviation with p-values calculated using a standard t-test.

| Gene | Forward Primer | Reverse Primer |
|---|---|---|
| GapDH | 5'-CAT GTT CGT CAT GGG TGT GAA CCA-3' | 5'-AGT GAT GGC ATG GAC TGT GGT CAT-3' |
| VIM | 5'- GAC CAG CTA ACC AAC GAC AAA GC -3' | 5'-CAG AGA CGC ATT GTC AAC ATC CTG -3' |
| CDH2 | 5'-GAG GAG TCA GTG AAG GAG TCA GC -3' | 5'- ATC AGA CCT GAT CCT GAC AAG CTC -3' |
| CDCA5 | 5'- CTG GCC GAA GAC ACC CAG T -3' | 5'- GTC CTC CTT AGT AAG CTC CCT GC -3' |
| RIOK1 | 5'- GCG GAC TCC TCT GAC AGT GAA AAC -3' | 5'- TCC AGA CAT AAC CCT TGG CGA G -3' |
| ADD1 | 5'- TGG TAT GGT GAC TCC TGT GAA CG -3' | 5'- GCT CGG AGT TCA CTC TGG TTG TG -3' |
| DBNL | 5'- GGG TTG GTA AAG ACA GCT TCT GG -3' | 5'- CAC ATG TTG ACC CCT GCT CAT TTC -3' |
| FLRT3 | 5'- CGT CTT CCT GGA GAT GCT CAG T -3' | 5'- GGT AGC TTC CGT TAC TTC AGA ACC -3' |
| BLVRB | 5'- ACT CCT CCA GGC TGC CAT C -3' | 5'- ATC ACT GTC GTG GGA CTG AGG -3' |
| AKR12C | 5'- GAC CCA GTC CTT TGT GCC TTG -3' | 5'-GGA ATT CAA ACA CCT GCA CGT TC -3' |

**Table S3: Sequences for primers used to validate RNA-sequencing data. Related to Figure 2 and Figure 4.**

**Alternative Splicing Validation**

Total RNA was isolated from cells using TriSure (Bioline, Luckenwalde Germany) and reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA), both according to the manufacturer's

recommendations. PCR was performed using Taq DNA polymerase (Invitrogen, Carlsbad, CA), and PCR products were visualized on a 1% agarose gel.

| Gene | Forward Primer | Reverse Primer | Description |
|---|---|---|---|
| CD44 | 5'- CGT GAT GGC ACC CGC TAT G -3'; Exon 5 | 5'- CTT CTT GAC TCC CAT GTG AGT GTC -3'; Exon 16 | CD44V- Exons 1-19=1451bp product<br>CD44E- 1-5, 12-19= 703bp product<br>CD44S- Exons 1-5, 15-19= 307bp product |
| CTNND1 | 5'- TTC TCA GCA CCT TGG CGA AG -3'; Exon 1 | 5'- CTG CAT CCT GGG GAT GGT G -3'; Exon 5 | Inclusion of exons 2 and 3 =664bp product |
| ENAH | 5'- GGC CTC TTC AAC AAG TAC ACC TG -3'; Exon 10 | 5'- CTG CTT CAG CCT GTC ATA GTC AAG -3'; Exon 12 | Exclusion of 63bp between exons 11 and 12= 169bp product |
| MYO1B | 5'- CCA GCA GAC AAA GAG TTC CGC -3'; Exon 21 | 5'- AGC TCC TTG TGA GTA GAA TCC AAG -3'; Exon 26 | Inclusion of exons 23 and 24= 501bp product |
| DLG1 | 5'- GGT CGG AGT GAT TCC CAG TAA AC -3'; Exon 17 | 5'- AGA CGT ATT CTT CTT GAC CAG GTA -3'; Exon 20-21 | Mutually exclusive exon 19= 66bp size difference |

**Table S4: Sequences for primers used to validate alternative splicing. Related to Figure 2.**

**UPLC-MS/MS:**

**Sample Preparation:** Cells were scraped in 250 µl of ammonium bicarbonate pH 8.0 (AmBic) containing 0.25% (w/v) acid labile surfactant (ALS-1). After freeze-thawing, samples were lysed by probe sonication (3 x 3 s) on ice. After centrifugation, protein concentration was determined by Bradford assay. 10µg of each sample was normalized to equal volumes with lysis buffer followed by addition of 10mM DTT. Samples were heated at 80 ºC for 10 min to denature and reduce the samples. After cooling to room temperature, samples were alkylated with 25mM iodoactamide (IAM). Excess IAM was quenched by addition of 10mM DTT. 1:25 (w/w) Sequencing Grade modified trypsin (Promega, Madison, WI) was added and samples were incubated overnight at 37ºC on a Thermomixer. After overnight digestions, ALS-1 was degraded by addition of trifluoroacetic acid (TFA) and acetonitrile (MeCN) to 1% and 2% (v/v) respectively, followed by heating at 60ºC for 2h and centrifuging to remove degraded ALS-1. Finally, 50 fmol/µl of trypsinized yeast ADH1 (Massprep; Waters Corporation, Milford, MA) was added and samples were transferred to Maximum Recovery LC vials (Waters).

**Quantitative Proteomic Analysis:** Quantitative one-dimensional LC-MS/MS was performed once per sample using 500ng of peptide digest. Samples were analyzed using a nanoACQUITY UPLC system (Waters) coupled to a Q Exactive HF Orbitrap high resolution accurate mass tandem mass spectrometer (Thermo) via a nanoelectrospray ionization source at Duke University's Proteomics and Metabolomics Core Facility. Briefly, the sample was first trapped on a Symmetry C18 300 µm x 20 mm trapping column (5 µl/min at 99.9/0.1 v/v H2O/MeCN for 5 min), after which the analytical separation was performed using a 1.7 µm ACQUITY HSS T3 C18 75 µm x 250 mm column (Waters) using a 90 min gradient of 5 to 30% MeCN with 0.1% formic acid at a flow rate of 400 nl/min with a column temperature of 55ºC. Data collection on the Q Exactive HF mass spectrometer was performed in a data-dependent MS/MS manner, using a 120,000 resolution precursor ion (MS1) scan followed by MS/MS (MS2) of the top 12 most abundant ions at 30,000 resolution. MS1 was accomplished using an automatic gain control (AGC) target of 3e6 ions and 50 msec maximum injection time. MS2 used AGC target of 5e4 ions and 45 ms maximum injection time, 1.2 m/z isolation window, 27 V normalized collision energy, and 20 s dynamic exclusion. The total analysis cycle time for each sample injection was approximately 2 h. Samples included two QC pool samples for column condition and additional QC pool samples analyzed at the beginning, middle and end of the study. Following the analyses, the data was imported into Rosetta Elucidator v4.0 (Rosetta Biosoftware, Inc.), and all LC-

MS files were aligned based on the accurate mass and retention time of detection ion ("features") using a PeakTeller algorithm (Elucidator). The relative peptide abundance was calculated based on area-under-the-curve (AUC) of aligned features across all runs. The dataset had 368,613 quantified features and HCD fragmentation was performed to generate approximately 892,447 MS/MS spectra for sequencing by database searching. This MS/MS data was searched against the reviewed (Swiss-Prot) protein sequences in the Uniprot (www.uniprot.org) database with Homo Sapiens taxonomy (downloaded on 08/16/16) as well as the study-specific proteins mouse p53DD, human R24C CDK4, T58C c-Myc and G12V H-ras, as well as the contaminant bovine serum albumin and surrogate standard yeast ADH1. In addition, the database contained an equal number of and reverse "decoy" protein sequences for false discovery rate determination (and a total of 40,407 entries). Amino acid modifications allowed in database searching included fixed carbamidomethyl on Cys, and variable deamidation of Asn/Gln, peptide N-terminal Gln->pyroGlu and protein N-terminal acetylation; variable modifications were deemed appropriate for these samples by a preliminary analysis using Byonic Preview (Protein Metrics, Inc). The data was searched with 7 ppm precursor, 0.02 Da product ion tolerance, and tryptic enzyme specificity, allowing up Project 1089: cancer vs normal HMECs to two missed cleavages. The data was annotated at 0.4% peptide FDR using the PeptideTeller algorithm in Rosetta Elucidator.

**Western blots**

Whole cell lysates were prepared by scraping into cold 0.1% SDS lysis buffer. Protein was cleared by centrifugation, quantified with a Bradford assay, and boiled in Laemmli loading buffer. ~20µg of protein was then size separated on a criterion SDS PAGE gel (Biorad, Hercules, CA, USA). Protein was transferred onto nitrocellulose membrane, and transfer was verified using Ponceau S staining. Membranes were blocked with 5% Non-fat milk in TBS+0.1% Tween20 (TBST) for one hour at room temperature, incubated with primary antibody at 4C overnight, washed thoroughly with TBST, incubated with secondary antibody for one hour at room temperature, washed extensively with TBST, and then developed using SuperSignal West Pico Chemiluminescent Substrate (ThermoFisher Scientific), and exposed to film. All primary antibodies are listed below. Anti-mouse (515-035-062) and anti-rabbit (111-035-003) HRP conjugated secondary were purchased from Jackson ImmunoResearch (West Grove, PA, USA) and used at a 1:20000 dilution.

| Gene | Antibody Source and catalog number | Dilution |
|---|---|---|
| GapDH | Santa Cruz; sc-47724 | 1:1000 |
| B-tubulin | Santa Cruz; sc-5274 | 1:1000 |
| VIM | Santa Cruz; sc-6260 | 1:250 |
| CDH2 | Santa Cruz; sc-271386 | 1:250 |
| CDCA5 | Santa Cruz; sc-365319 | 1:200 |
| RIOK1 | Proteintech; 17222-1-AP | 1:500 |
| ADD1 | Santa Cruz; sc-33633 | 1:200 |
| DBNL | Santa Cruz; sc-398498 | 1:200 |
| FLRT3 | Santa Cruz; sc-514482 | 1:200 |
| BLVRB | Santa Cruz; sc-373692 | 1:200 |
| AKR1C2 | Iowa Hybridoma Bank | 0.375µg/ml |

**Table S5: Primary antibodies used for validation Western blots. Related to Figure 4.**

**ADD1 shRNA knockdown:**

HEK-293T cells were grown to ~70% confluency and co-transfected with 1µg shRNA lentiviral plasmid (see table), 900ng psPAX2 packaging plasmid (a gift from Didier Trono; Addgene plasmid #12260), and 100ng pMD2.G envelope plasmid (a gift from Didier Trono; Addgene plasmid #12259) using FuGENE-6 transfection reagent (Promega, Madison, WI). Media was changed to DMEM+30%FBS 16 hours post-transfection. Virus-containing supernatant was harvested 48 and 72 hours post-transfection and filtered with a 45µM filter.

Hexadimethrine bromide (Sigma-Aldrich, St. Louis, MO) was added to 8µg/ml final concentration, and 0.5ml of prepared viral supernatant was added to IMO cells and incubated for 48 hours. ADD1 knockdown was assessed with a western blot.

| TRC_ID (plate map) | Olig_Seq | Gene | Refseq |
|---|---|---|---|
| | CCGGGCGCGATAGCGCTAATAATTTCTCGAGAAATTATTAGCGCTATCGCGCTTTTTG | Neg ctrl shRNA | Non-targeting sequence |
| 84018 | CCGGGCAGGTTTACAATTTAGCTTACTCGAGTAAGCTAAATTGTAAACCTGCTTTTTG | ADD1 (shRNA 1) | NM_001119.3,NM_014189.2, NM_014190.2,NM_176801.1 |
| 84019 | CGGCGGTGTAAATTGGCAGCGTTTCTCGAGAAACGCTGCCAATTTACACCGTTTTTG | ADD1 (shRNA 2) | NM_001119.3,NM_014189.2,NM_014190.2,NM_176801.1 |
| 84020 | CCGGGCAGGAATTTGAAGCCCTCATCTCGAGATGAGGGCTTCAAATTCCTGCTTTTTG | ADD1 (shRNA 3) | NM_001119.3,NM_014189.2,NM_014190.2,NM_176801.1 |

**Table S6: TRC1 lentiviral shRNA clones. Related to Figure 4.**

**Data and Software Availability**

All data has been deposited in GEO and can be accessed by GEO series accession number GSE110677.

**Supplemental References:**


Bisogno, L.S., and Keene, J.D. (2017). Analysis of post-transcriptional regulation during cancer progression using a donor-derived isogenic model of tumorigenesis. Methods *126*, 193-200.

Counter, C.M., Hahn, W.C., Wei, W., Caddle, S.D., Beijersbergen, R.L., Lansdorp, P.M., Sedivy, J.M., and Weinberg, R.A. (1998). Dissociation among in vitro telomerase activity, telomere maintenance, and cellular immortalization. Proc Natl Acad Sci U S A *95*, 14723-14728.

Eden, E., Lipson, D., Yogev, S., and Yakhini, Z. (2007). Discovering motifs in ranked lists of DNA sequences. PLoS Comput Biol *3*, e39.

Eden, E., Navon, R., Steinfeld, I., Lipson, D., and Yakhini, Z. (2009). GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. BMC Bioinformatics *10*, 48.

Hahn, W.C., Dessain, S.K., Brooks, M.W., King, J.E., Elenbaas, B., Sabatini, D.M., DeCaprio, J.A., and Weinberg, R.A. (2002). Enumeration of the simian virus 40 early region elements necessary for human cell transformation. Mol Cell Biol *22*, 2111-2123.

Irimia, M., Weatheritt, R.J., Ellis, J.D., Parikshak, N.N., Gonatopoulos-Pournatzis, T., Babor, M., Quesnel-Vallieres, M., Tapial, J., Raj, B., O'Hanlon, D*., et al.* (2014). A highly conserved program of neuronal microexons is misregulated in autistic brains. Cell *159*, 1511-1523.

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol *14*, R36.

Trapnell, C., Williams, B.A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M.J., Salzberg, S.L., Wold, B.J., and Pachter, L. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol *28*, 511-515.

Yeh, E., Cunningham, M., Arnold, H., Chasse, D., Monteith, T., Ivaldi, G., Hahn, W.C., Stukenberg, P.T., Shenolikar, S., Uchida, T*., et al.* (2004). A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. Nat Cell Biol *6*, 308-318.