SUPPLEMENTAL MATERIALS AND METHODS

TOMiD analysis

For each sample, 100-nt sequence reads were obtained utilizing the Illumina HiSeq3000 instrument at the University of Florida Interdisciplinary Center for Biotechnology Research. Sequences were transferred and stored on UF's HiPerGator High-Performance Computing Environment and all subsequent analysis steps were performed on this system. Read quality control and analysis were first performed using FastQC (version 0.11.7) (Andrews 2010). Trimmomatic (version 0.36) was then utilized to remove adapter sequences and low-quality bases (PHRED <= 20) from read ends, with reads below 26 bases in length discarded from further analysis (Bolger et al. 2014). Reads from both mate pairs remaining after trimming were merged using PEAR (version 0.9.6) (Zhang et al. 2014) and the merged sequence files were combined with any unpaired reads resulting from trimming. Reads were sequenced utilizing a 4-nucleotide barcode to distinguish between PCR-duplicate and identical disparate reads. As such, PCR-duplicates were removed using fastx-collapser (fastx_toolkit, version 0.0.14) (Gordon and Hannon 2010) and barcodes were removed using cutadapt (version 1.8.1) (Martin 2011). Reads were then again collapsed using fastx-collapser, with count information tallying unique sequence reads encoded within read sequence identifiers (Gordon and Hannon 2010).

```
# FastQC Analysis:
$ fastqc -q --noextract --threads ${THREADS} ${READS_R1} ${READS_R2}

# Trimmomatic Trimming:
$ trimmomatic PE -threads ${THREADS} ${READS_R1} ${READS_R2} -baseout \
  ${OUT_DIR}/{READS_NAME}.fastq ILLUMINACLIP:{WD}/reads_raw/RPI_trimmomatic.fasta:
  2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:26

# Mate Pair Merging via PEAR
$ pear -f ${READS_1P} -r ${READS_2P} -o ${OUT_NAME_MGD} -j ${THREADS} \
  -y ${MEM_TOTAL}M -n 26

# Collapsing of PCR-Duplicates via FASTX_Collapser:
$ fastx_collapser -v -i ${OUT_NAME_MGD} -o ${OUT_NAME_PCR_DEDUP}

# Removal of Read Barcodes via cutadapt
$ cutadapt -u 4 -u -4 -m 18 -o ${OUT_NAME_PCR_DEDUP_CUT} ${OUT_NAME_PCR_DEDUP}

# Collapsing of Quantitative Duplicates via FASTX_Collapser:
$ fastx_collapser -v -i ${OUT_NAME_PCR_DEDUP_CUT} \
  -o ${OUT_NAME_PCR_DEDUP_CUT_QUANT_DEDUP}
```

For alignment of primary target sequences, the hOH7 reference sequence database included with the Hyb software package (version 20141126) (Travis et al. 2014) was utilized and an alignment reference index was created using Bowtie2-build (version 2.3.4.3) (Langmead and Salzberg 2012). Reads were aligned to the reference genome using Bowtie2 with parameters designed to allow local alignment of short segments of the reads to multiple reference sequences to identify possible read subsequences (Langmead and Salzberg 2012). Alignments were then sorted by query read name using Samtools sort (version 1.9) (Li et al. 2009).

```
# Alignment of Reads for Candidate Hybrid Identification
$ bowtie2 -f ${OUT_NAME_PCR_DEDUP_CUT_QUANT_DEDUP} -x ${INDEX_FILE} \
  --threads ${THREADS} -S ${RAW_OUT_SAM} -D 20 -R 3 -N 1 -L 16 -k 20 --local -i S,1,0.50 \
  --score-min L,18,0 --ma 1 --np 0 --mp 2,2 --rfg 5,1

# Sorting of Alignments:
$ samtools sort -n -o ${SORT_OUT_SAM} --threads ${THREADS} ${RAW_OUT_SAM}
```

Aligned reads for each sample were evaluated as potential hybrid sequences using a custom script implemented in Python3 using the pysam Python module with Samtools (Li et al. 2009; Heger et al. 2018; Van Rossum and Python Development Team 2018). Alignments were first evaluated on a per-read basis ignoring all full-length alignments. For reads with partial-length alignments, the longer alignment for each read was selected as the most likely subsequence to represent the "target" of a chimeric RNA molecule. Reads with partial alignments were then identified as a candidate hybrid if the length of the unaligned portion of the sequence is between 18 and 23 bases and were then separated into a candidate miRNA (cmiRNA) and target sequence from the unaligned and aligned portions, respectively. The Gibbs Free Energy of hybrid formation between each pair of sequences was predicted using UNAFold hybrid-min (version 3.8) (Markham and Zuker 2008), with only candidate sequences with a predicted free energy value lower than -7.0 kcal/mol retained. Finally, each exact candidate miRNA sequence was required to have been called identically in two or more reads to reduce the effect of ligation and sequencing error on cmiRNA identification.

```
# Analysis of Candidate Hybrids
$ CLASH_split_v10.py ${SORT_OUT_SAM} -o ${OUT_BASE} --script_mode --verbose
  --max_fold_energy "-7.0" --min_candidate_cov "2" --min_candidate_len "18" \
  --max_candidate_len "23" --action_log --name_prefix ${READS_NAME}_cmiRNA_ \
  > ${OUT_BASE}.out"

# Prediction of Free Energy of Folding with UNAFold hybrid-min
$ hybrid-min -o ${OUT_BASE}.fold.UNAFold ${OUT_BASE}_5p.fasta ${OUT_BASE}_3p.fasta
```

All cmiRNA molecules identified in sequences from each individual sample were then combined, duplicates were removed, and each candidate was assigned a unique identifier using a custom script implemented in Python3 (Van Rossum and Python Development Team 2018). Each cmiRNA was required to exist in more than one sample for further processing, giving a total requirement of >= 4 total unique hybrids required for each cmiRNA to pass filtration. For identification of cmiRNA transcript source, the RefSeq human RNA reference library was acquired from ftp://ftp.ncbi.nlm.nih.gov/refseq/H_sapiens/annotation/GRCh38_latest/refseq_identifiers/GRCh38_latest_rna.fna.gz on 2019-03-19 and an alignment reference index was prepared via Bowtie2-build (version 2.3.4.3) (Langmead and Salzberg 2012; O'Leary et al. 2016). The cmiRNA sequences were then aligned to the prepared reference transcriptome via Bowite2 (version 2.3.4.3) (Langmead and Salzberg 2012) and sorted via Samtools sort (version 1.9) (Li et al. 2009). A custom Python script utilizing pysam and Samtools was then used to annotate the candidate miRNA in each sample's respective candidate hybrid sequences, and final hybrids were output in the Hyb file format (Li et al. 2009; Travis et al. 2014; Heger et al. 2018; Van Rossum and Python Development Team 2018). Subsequent steps for visualization and quantification of identified cmiRNA and hybrid counts were then performed using additional scripts.

```
# Combination of Sample-Specific cmiRNAs
$ CLASH_combine_v02.py ${IN_FASTAS} --out_name_prefix ${READS_NAME} \
  -o ${OUT_BASE} --verbose --min_fasta_cov 2

# Alignment of Combined cmiRNAs to RefSeq Database
$ bowtie2 -f ${CMB_FASTA} -x ${INDEX_FILE} --threads ${THREADS} \
  -S ${OUT_SAM_RAW} -D 20 -R 3 -N 1 -L 16 --local -i S,1,0.50 --score-min L,18,0 \
  --ma 1 --np 0 --mp 2,2 --rfg 5,1
```

```
# Sorting of Alignments by Name
$ samtools sort -n -o ${OUT_SAM_SORT} --threads ${THREADS} ${OUT_SAM_RAW}"

# Assignment of cmiRNA to Alignments
$ CLASH_assign_v03.py ${IN_HYB} -s ${OUT_SAM_SORT} -v --action_log \
  -c ${CMB_CONTAINS} -o ${OUT_ASSIGN_BASE} --exclude_unclustered
```

TOMiD sensitivity analysis

To assess the sensitivity of the TOMiD in hybrid read detection, a subset of a previous qCLASH experimental dataset published by Gay et al. (Gay et al. 2018) was analyzed via both the standard Hyb chimeric read caller and by the TOMiD approach (Travis et al. 2014). Data from one experimental replicate of the previous dataset was first selected (WT_BR3). For identification of hybrid reads by Hyb, read preprocessing and calling of chimeras was performed as previously described by Gay et al. (Gay et al. 2018). The comprehensive output file containing the sequences of all hybrids identified by Hyb in FASTA format was then utilized for further analyses (WT_BR3_comp_hOH7_KSHV_hybrids.fasta).

To quantify the number of hybrids predicted by the target-oriented approach compared to Hyb, alignment of the previously identified hybrid sequences in FASTA format was first performed to the hOH7 reference database included with Hyb (version 20141126). The Bowtie2 read aligner (version 2.3.4.3) was employed using settings customized for short-read alignment (Langmead and Salzberg 2012; Travis et al. 2014). An initial implementation of the hybrid-identification portion of the TOMiD method ("CLASH-Split_v02.py") was then utilized for hybrid read prediction (for further details on the implementation of TOMiD, see the "TOMiD Analysis" section above). cmiRNA length requirements were each set to more permissive values to enable direct comparison of hybrid recapture between methods (minimum cmiRNA length: 17, maximum cmiRNA length: 24). A custom script written in Python3 ("fasta_compare_v02.py") was then utilized to compare the counts of identifiers of sequences present in both the initial hybrid FASTA file and the FASTA for hybrids predicted by TOMiD (Van Rossum and Python Development Team 2018). The resulting overlapping and independent sequence counts were plotted by a custom script written in the R Language (version 4.0.4) using the VennDiagram package (version 1.6.20) with colors selected using ColorBrewer 2.0 (Harrower and Brewer 2003; Chen and Boutros 2011; R Core Team 2012).

```
# Alignment of Hyb-Identified Hybrid Sequences to the hOH7 reference database
$ bowtie2 -f ${IN_FASTA} -x ${BT2_INDEX} --threads ${THREADS} -S ${OUT_SAM} \
  -D 20 -R 3 -N 0 -L 16 -a --local -i S,1,0.50 --score-min L,18,0 --ma 1 --np 0 --mp 2,2 --rfg 5,1

# Split alignments with prototype TOMiD Implementation:
$ CLASH_split_v02.py ${OUT_SAM} -o ${SPLIT_OUT_BASE} \
  --allow_miRNA_ref --allow_primary_miRNA_ref \
  --min_candidate_len 17 --max_candidate_len 24

# Quantify FASTA files:
$ fasta_compare_v02.py ${SPLIT_OUT_BASE}.cseq.fasta -f ${IN_FASTA} \
  -o ${COMPARE_BASE} -m seq_names \
  -v --write_output_file --write_output_summary --write_csv_summary

# Plot Venn Diagram
$ Plot_Venn.R
```

Identification of Drosha-independent miRNAs

For identification of possible Drosha-independent miRNAs, the counts of each detected cmiRNA within a unique hybrid were compared across the WT, Drosha-KO and Dicer-KO qCLASH datasets. The count of each cmiRNA per sample was first transformed into a proportion of that cmiRNA per sample by dividing the cmiRNA count by the total sample candidate hybrid count to normalize to the number of possible hybrids. A cutoff threshold was then applied retaining cmiRNA where $prop_{cmiRNA} \geq 1.0 * 10^{-5}$ (approximately 25 average reads/sample) to reduce the effect of large variations due to small sample counts, resulting in 672 remaining cmiRNA alignments. The qCLASH experiments providing sequencing data were conducted in two sets of two technical replicates. To reflect this distribution, category proportional count averages were first calculated across technical replicates per sample, and then again across different samples.

For each cmiRNA, Dicer-dependence was calculated as the proportion of the count of a specific miRNA in the Dicer-KO to its count in WT, and the Drosha-dependence was similarly calculated as the proportion in Drosha-KO compared to WT. A minimum Drosha-dependence score of $dep_{Drosha} \geq 1.5$ was set to require enrichment of a cmiRNA in the Drosha-KO condition. Although many cmiRNA had zero counts in the Dicer-KO condition, and thus a Dicer-Dependence

score of $dep_{Dicer} = 0.00$, a minimum Dicer-dependence value of $dep_{Dicer} = 0.01$ was utilized in subsequent calculations to prevent errors from division by zero. A Drosha-independent miRNA discovery scoring metric was then calculated by dividing the Drosha-dependence by the Dicer-dependence, with a high score indicating strong Drosha-independence and Dicer-dependence for a given cmiRNA (Equations 1-3). A $score_{Drosha/Dicer} \geq 20$ was chosen as a cutoff threshold to focus the analysis on the highest-scoring candidates (range 0 to 501).

$$prop_{cmiRNA} = \frac{count_{cmiRNA}}{count_{total}}$$

Equation 1: Calculation of cmiRNA Sample Proportion

$$dep_{DRO} = \frac{prop_{DRO}}{prop_{WT}}, \qquad dep_{DI} = \frac{prop_{DI}}{prop_{WT}}$$

Equation 2: Calculation of cmiRNA Drosha-Dependence and Dicer-Dependence

$$score_{Drosha/Dicer} = \frac{dep_{DRO}}{\max(dep_{DI}, 0.01)}, \qquad with\ dep_{DRO} \geq 1.5$$

Equation 3: Calculation of combined Drosha/Dicer Site score from Drosha-Independence and Dicer-Dependence scores

Identification of miR-snaR binding sites

To identify the miR-snaR binding site(s) in all targets, the miR-snaR sequence: "AGCCTGGTCCACATGGGTCGGA" was added to hOH7 genome database to create a customized database. Processed reads obtained from HCT116 WT and Drosha-KO cells were generated as mentioned above. Experimental CLASH Reads from 293 cells were downloaded from the GEO database (GSE46039) and processed as previously (Helwak et al. 2013). We utilized Hyb (version 20141126) for read alignment, including extension of target sequences by 25 nucleotides where possible to ensure that the miR-snaR binding site is completely contained within hybrid reads (Travis et al. 2014). Results of Hyb analysis include a file in the viennad format described by Travis et al., containing: the base-pairing pattern of the miRNA/target pair, the nucleotide position and sequence of the target from the hOH7 database, and a predicted intra-hybrid folding energy. The specific target nucleotide position was extracted from viennad files for further analysis. The miR-snaR target sites that included seed-region complementarity and which appeared in at least two biological replicates were then identified and selected for further analysis (Fig. 4B). The start and end positions of the target are defined by overlapping read sequences mapping to the

same site. We then composed a custom Python 2.7 script ("Viennad_Call_Peak.py") to call miR-snaR binding peak region in three datasets (HCT116 WT, HCT116 Drosha-KO and 293 cells) based on the viennad files.

```
# Align processed reads into hOH7 genome file and extend each target coordinates by 25 nucleotides
$ hyb analyse fold=UNAfold in=${IN_FASTQ} db=${HOH7_GENOME} type=mim pref=mim

# Identify miR-snaR binding site(s)
$ python2 Viennad_Call_Peak.py -i ${VIENNAD_FILES} -g ${HOH7_GENOME} \
  -r ${REPLICATE_FILE} -m {MIRNA_NAME} -o ${OUTPUT_NAME}
```

miRNA/target interactions analysis

To analyze the miR-snaR/target base-pairing pattern, the merged reads generated as described above were first filtered to contain only those with an alignment to miR-snaR utilizing Bowtie2 (Langmead and Salzberg 2012). Using the database containing the annotated miR-snaR as described above, Hyb was then utilized to identify CLASH hybrids from the filtered reads, including the use of UNAFold hybrid-min (version 3.8) for prediction of folding patterns (Markham and Zuker 2008; Travis et al. 2014). A Python3 script ("hybkit_analyze_miR-snaR_folding.py") built on the hybkit API (version 0.2.1a) was utilized to filter reads containing mRNA or mRNA-pseudogene targets and calculate the per-base folding characteristics of miR-snaR (Stribling 2020).

```
# Alignment/Selection of only miR-snaR-containing Reads:
$ LANG=C bowtie2 -f ${IN_FASTA} -x ${MIR_SNAR_INDEX_FILE} --threads ${THREADS} \
  -S ${OUT_SAM} --al ${OUT_FASTA} --norc -a -R 3 -N 0 -L 16 --local -i S,1,0.50 \
  --score-min L,18,0 --ma 1 --np 0 --mp 2,2 --rfg 5,1 --no-unal

# Hyb miR-snaR Hybrid Identification
$ LANG=C BOWTIE2_PARAM="-D 20 -R 3 -N 1 -L 16 -a --local -i S,1,0.50 \
  --score-min L,18,0 --ma 1 --np 0 --mp 2,2 --rdg 5,1 --rfg 5,1 -p ${THREADS}" \
  HYB_DB=${HYB_DB_DIR} hyb detect align=bowtie2 word=11 format=fasta \
  analyse fold=UNAfold in=${IN_FILE} db=${DB_NAME}

# Analyze miR-snaR Folding Pattern with Hybkit
$ python3 hybkit_analyze_miR-snaR_folding.py
```

To analyze the base pairing pattern of all miRNAs, reads merged as described above were utilized directly with Hyb and UNAFold using the miR-snaR-augmented database (Markham and Zuker 2008; Travis et al. 2014). A Python3 script ("hybkit_analyze_other_folding.py") built on the hybkit API (version 0.2.1a) (Stribling 2020) was utilized to filter hybrids to only miRNA-containing hybrids without miR-snaR with targets of mRNAs or mRNA-pseudogenes. The per-base folding characteristics all non-miR-snaR miRNAs in the dataset was then calculated.

```
# Hyb Hybrid Identification
$ LANG=C BOWTIE2_PARAM="-D 20 -k 200 -R 3 -N 1 -L 16 --local -i S,1,0.50 \
  --score-min L,18,0 --ma 1 --np 0 --mp 2,2 --rdg 5,1 --rfg 5,1 --norc -p ${THREADS}" \
  HYB_DB=${HYB_DB_DIR} hyb detect align=bowtie2 word=11 format=fasta analyse \
  fold=UNAfold in=${IN_FILE} db=${DB_NAME}"

# Analyze non-miR-snaR Folding Pattern with Hybkit
$ python3 hybkit_analyze_other_folding.py
```

***In vitro* transcription of snaR-A**

To generate DNA templates, PCR reactions were performed in 50 µL with 1 unit of Phusion polymerase (NEB) with High Fidelity buffer, 40 nM forward and reverse primers, 200 nM dNTP, and 20 ng pBS-snaR-A. In a thermal cycler, repeat (98°C for 15s, 60°C for 20s, 72°C for 20s) for 35 cycles after initial denaturing at 98°C for 30s. The DNA products were separated on a 3% agarose gel and then extracted using gel extraction kit (ZYMO research). Concentrations of PCR DNA were measured by Nanodrop. In a 20 µL in vitro transcription reaction, 250 ng of PCR DNA template, 20 U of Murine RNase inhibitor (NEB), 5 U of T7 RNA polymerase, 1mM (each) of NTP were incubated in 1X transcription buffer [40 mM Tris-HCl pH8.0, 25 mM NaCl, 2 mM spermidine$(HCl)_3$ 8 mM $MgCl_2$ and 10 mM DTT] at 37°C overnight and separated on a 6% denaturing PAGE gel. Extracted gels were soaked in RNA elution buffer (0.3 M NaOAc and 25 mM Tris) and incubated at room temperature overnight with gentle agitation. RNAs were then PCA extracted from the elution buffer and measured concentration with Nanodrop.

***In vitro* Dicer cleavage assay**

Briefly, Flag-tagged human Dicers were overexpressed by transfection of the expression plasmid in 293T cells and purified by α-Flag antibodies. In cleavage assay, approximately 20 ng

gel purified RNA was mixed with 2 µl 10 X buffer (200 mM Tris pH 6.5, 15 mM $MgCl_2$, 250 mM NaCl, 10 mM DTT and 10% glycerol), approximately 100 ng Dicer on beads, and water was added to 20µl total volume. The mixture was incubated at 37°C for 1 hour with gentle agitation. The RNA was then PCA extracted and subjected to northern blot analysis.

Plasmid and miRNA mimic transfection

Plasmid DNA was transfected using polyethylenimine (PEI). 293T cells were seeded 24 hours prior to transfection. PEI and plasmid DNA were separately diluted in OPTI-MEM (Gibco) and incubated at room temperature for 5 minutes. Diluted PEI and DNA were then combined and incubated at room temperature for an additional 15 minutes before being applied to cells. Cells were then incubated at 37°C. Cell medium was changed 24 hours post-transfection. Total RNA was collected 48 hours post-transfection. For transfection in 24-well plates, 1 µg of plasmid DNA and 4 µg of PEI were used per well. For 6-well plates, 2 µg of DNA and 8 µg of PEI were used per well.

miRNA mimic was transfected using Lipofectamine RNAiMAX transfection reagent. 293T, MCF-7, and MDA-MB-231 cells were seeded immediately prior to transfection. RNAiMAX and miRNA mimic were separately diluted in OPTI-MEM and incubated at room temperature for 5 minutes. Diluted RNAiMAX and miRNA mimic were combined and incubated at room temperature for an additional 15 minutes before being applied to cells. Cells were returned to incubation at 37°C and harvested 48 hours post-transfection. For 6-well plates, 30 pmol of miRNA mimic and 9 µl of RNAiMAX were used per well. For 24-well plates, 5 pmol of miRNA mimic and 1.5 µl of RNAiMAX were used per well.

Ago immunoprecipitation

α-Ago antibody (clone 4F9) was used for Ago-IP from 293T, MCF-7, MDA-MB-231 and HCT116 cells (Ikeda et al. 2006). Approximately 1 million cells were lysed by 200 µL of NP-40 lysis buffer (50 mM Tris–HCl pH 7.5, 1% NP-40, 10% glycerol, 150 mM NaCl, 5 mM EDTA, and 0.5 mM PMSF) for 30 minutes at 4°C and supernatant was collected by centrifugation at 21,000 × g for 10 minuets at 4°C. Total protein concentration in the supernatant was measured by DC protein assay kit (Bio-Rad). Total protein lysate was subjected to immunoprecipitation with α-Ago antibody-coated Protein L beads (Pierce, SE251848) for 2 hours at 4°C. After incubation,

the beads were washed twice with cold Buffer D (20 mM HEPES pH 7.9, 10% glycerol, 0.1 M KCl, 1 mM EDTA, 0.1% NP-40 and 0.5 mM PMSF). RNAs were PCA extracted and subjected to northern blot analysis.

Synthetic miRNA mimic

Synthetic mature miRNA and miRNA* were obtained from Integrated DNA Technologies (IDT) and annealed to generate miRNA duplexes. The synthetic sequences were designed to contain a 5' phosphate on the mature miR-snaR strand (indicated by "/phosphate/") and a 2-nucleotide 3' overhang. miR-snaR and miR-snaR* were combined with siRNA annealing buffer (100 mM potassium acetate, 2 mM magnesium acetate, 30 mM HEPES-KOH pH 7.4) and incubated for 1 minute at 90°C followed by 1 hour at 37°C. The final concentration of annealed miRNA was 20 μM. The negative control miRNA and miR-snaR antagomir (inhibitor) were designed and synthesized by GenePharma.

Western blotting

Total protein lysate was separated using 10 or 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Contents of the SDS-PAGE were transferred to nitrocellulose or PVDF membrane using LifeTech transfer module at 0.3 Amp for one hour. The membrane was then incubated with 5% milk in 1X TBST (150 mM NaCl, 20 mM Tris pH 7.5, 0.1% Tween-20) for one hour at room temperature. Next, the membrane was divided, and appropriate sections were incubated with antibody against NME1 (1:1000 diluted in 5% milk) or control proteins (GAPDH or Hsc70, 1:10,000 diluted in 5% milk) at 4°C overnight. Membrane sections were then washed 5 times with 1X TBST for 5 minutes each. Membrane sections were then incubated with HRP-conjugated secondary antibody IgG (1:5000 – 1:10,000) in 5% milk in 1X TBST buffer for one hour at room temperature. This was followed by washing with 1X TBST five times for five minutes each. Finally, membrane sections were incubated with enhanced chemiluminescence (ECL) reagent mixture (1 part oxidizing reagent:1part luminol reagent) (Perkin Elmer) for 1 minute before being imaged on the Bio-Rad ChemiDoc imager.

Northern blotting

10 – 20 µg of total RNA from each sample was separated using 15% Urea-PAGE. Contents of the Urea-PAGE were transferred to Hybond N+ membrane (GE) using LifeTech transfer module at 0.2 Amp for one hour. The membrane was crosslinked twice using 254 nm UV crosslinker at 120 mJ/cm^2. In the hybridization oven, membrane was incubated with 10 ml ExpressHyb hybridization solution (Takara) in a hybridization tube for 30 minutes at 30°C. Membrane was then hybridized overnight at 30°C using probes conjugated with IR-dye (Table S1). Membrane was then washed twice. The first wash used 2X saline-sodium citrate (SSC)-0.1% SDS wash buffer. The second wash used 1X SSC-0.1% SDS buffer. For both washes, membrane was shaken at 110 rpm for 10 minutes at room temperature. Following washes, membrane was scanned on Amershan Typhoon scanner (GE health) to detect emission at 600 nm and 800 nm.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Sensitivity of the Target Oriented miRNA Discovery (TOMID) method in hybrid prediction compared to Hyb. Venn diagram depicting the overlap of chimeric sequence reads (hybrids) identified by the standard Hyb method and those predicted by TOMiD from a sample of a previously-published Quick Crosslinking and Sequencing of Hybrids (qCLASH) dataset (WT_BR3; Gay et al. 2018). Of the 804,314 sequences identified as hybrids by Hyb, TOMiD predicts 737,434 (91.7%; Blue) of these sequences as hybrids, with 66,880 (8.32%; Red) sequences identified by Hyb only. Investigation of the source of these differences suggests that hybrid reads assigned only by Hyb arise due to an increased stringency of the assumptions in TOMiD analysis where overlaps between alignments are not considered.

Supplemental Figure 2. Mapping the 5' and 3' ends of snaR-A transcripts. Sequencing chromatogram of PCR products from 3' RACE (**A**) and 5' RACE (**B**) of snaR-A. (**C**) Schematic of sequencing results of individual clones obtained from 5' RACE of snaR-A.

Supplemental Figure 3. SnaR-A and miR-snaR expression levels in HEK293T Drosha-KO and HCT116 cells. (**A**) Northern blot detection of snaR-A and U6 in total RNAs extracted from cells transfected with pBS or pBS-snaR-A. (**B**) Read number of miR-snaR in small RNA seq from HCT116 cells.

Supplemental Figure 4. Multiple sequence alignment for the NME1 3' UTR in different primate species. The sequence that is fully complementary with miR-snaR seed sequence is indicated by the bracket at the bottom. In the available representatives of the higher-order primates comprising the Homininae subfamily: *Homo* (humans), *Gorilla* (gorillas), and *Pan* (chimpanzees and bonobos), absolute conservation of the complete miR-snaR binding site is observed (*Homo sapiens*, *Gorilla gorilla gorilla*, and *Pan paniscus)*. Absolute site conservation is also observed in *Pan troglodytes* (NM_001204512.1; not shown).

Supplemental Figure 5. miR-snaR does not influence cell proliferation. (**A**) Western blots show reduction of NME1 protein in 293T and MDA-MB-231 cells after transfection of miR-snaR mimic with GAPDH as a loading control. Asterisk (*) marks a non-specific band. (**B**) Left: Western blots show reduction of NME1 protein in MCF-7 cells with Hsc70 as a loading control. Asterisk (*) marks a non-specific band. Right: Quantification of three experiments as shown left. (Student's t test, **$p \leq 0.01$). (**C**) Bar graph of MTT assay measurements (absorbance measured in the range of 570-690 nm) using cells transfected with either a control or a miR-snaR mimic. Error bars represent standard deviation from three experiments. Differences are not statistically significant (n.s., Student's t test, $p > 0.05$).

Supplemental Table 1. Sequences of oligonucleotides used in this study.

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Figure S1

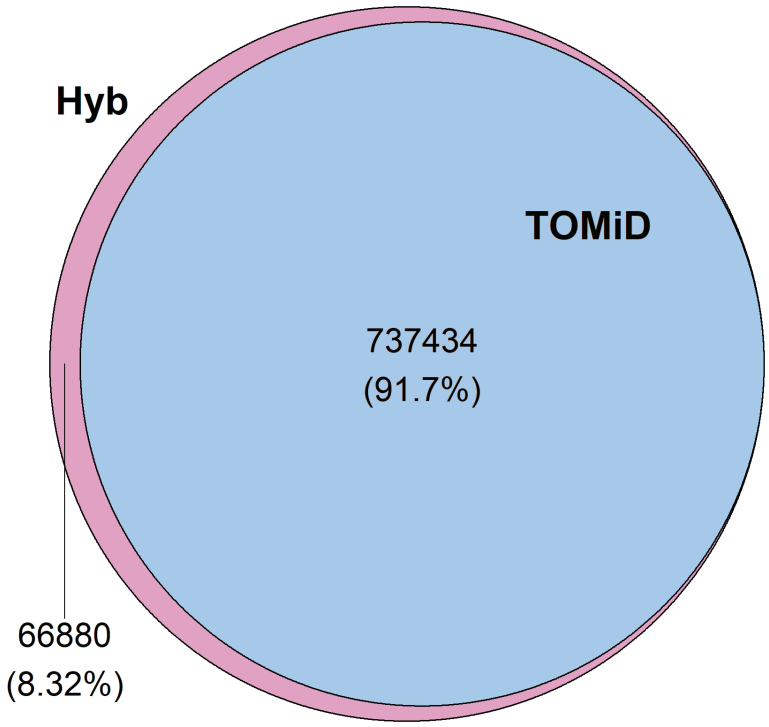
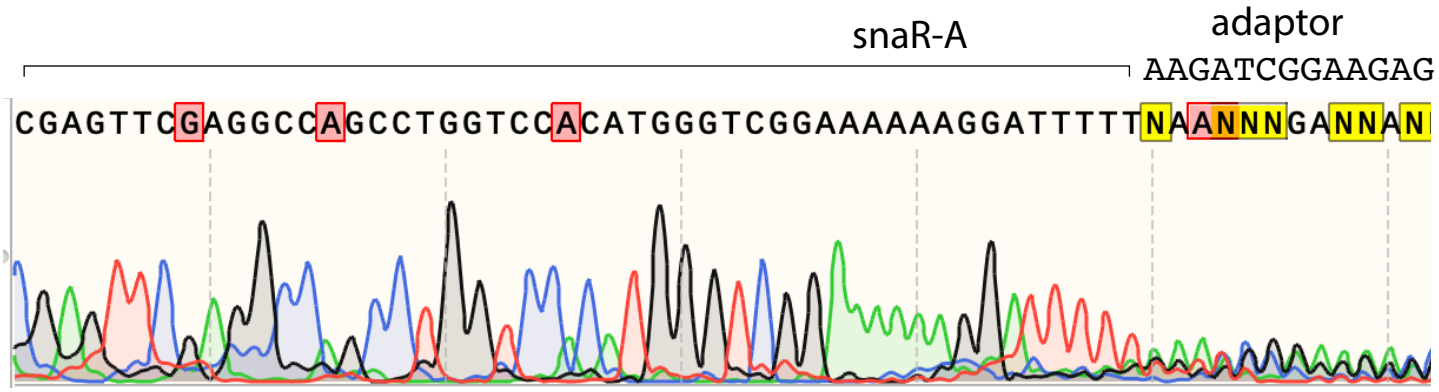
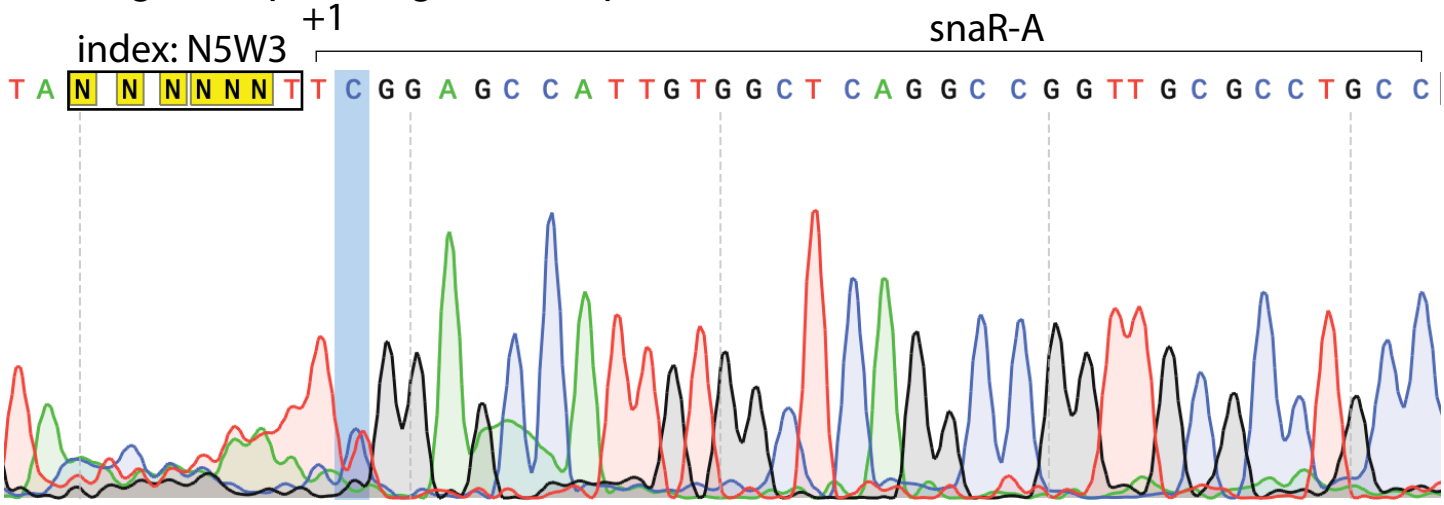


Figure S2

A Sanger sequencing of PCR products from 3′ RACE



B Sanger sequencing of PCR products from 5′ RACE



C Sanger sequencing of individual clones from 5′ RACE

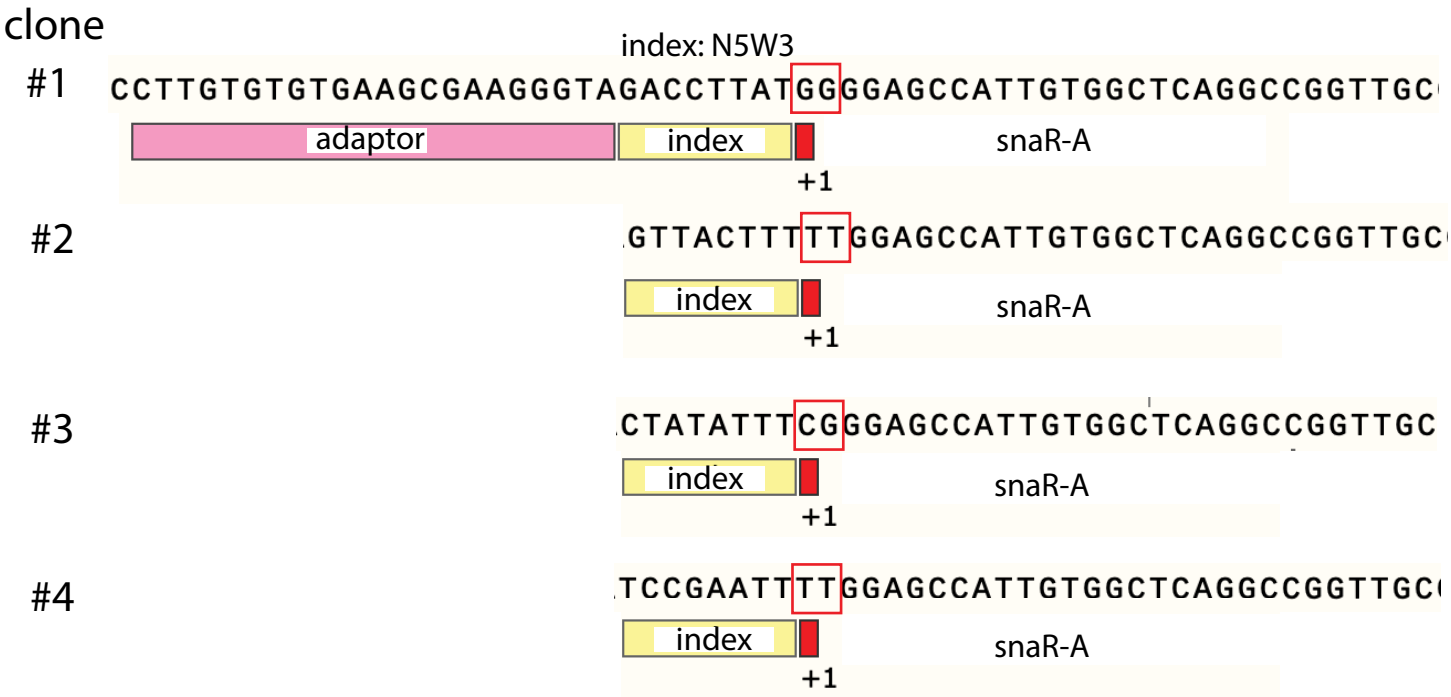
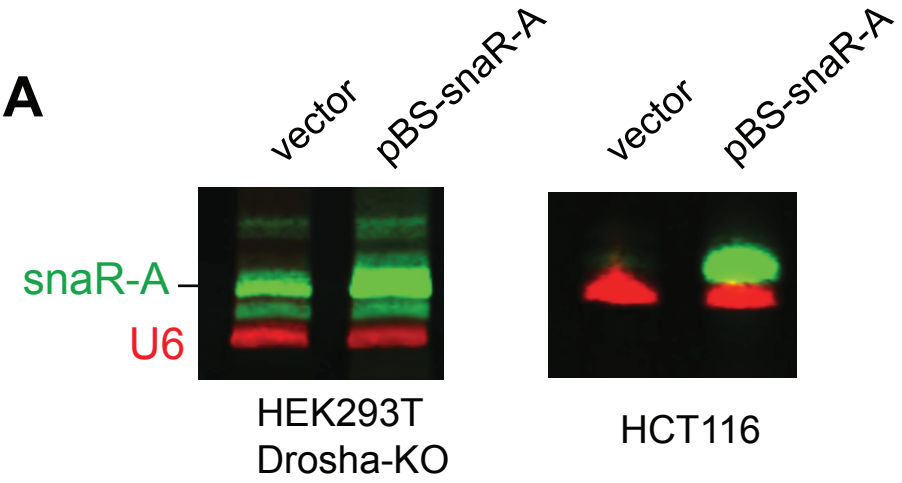


Figure S3

A



B

HCT116 cells	miR-snaR read #
WT	322
Drosha-KO	26,181
Dicer-KO	2
XPO5-KO	49

Figure S4

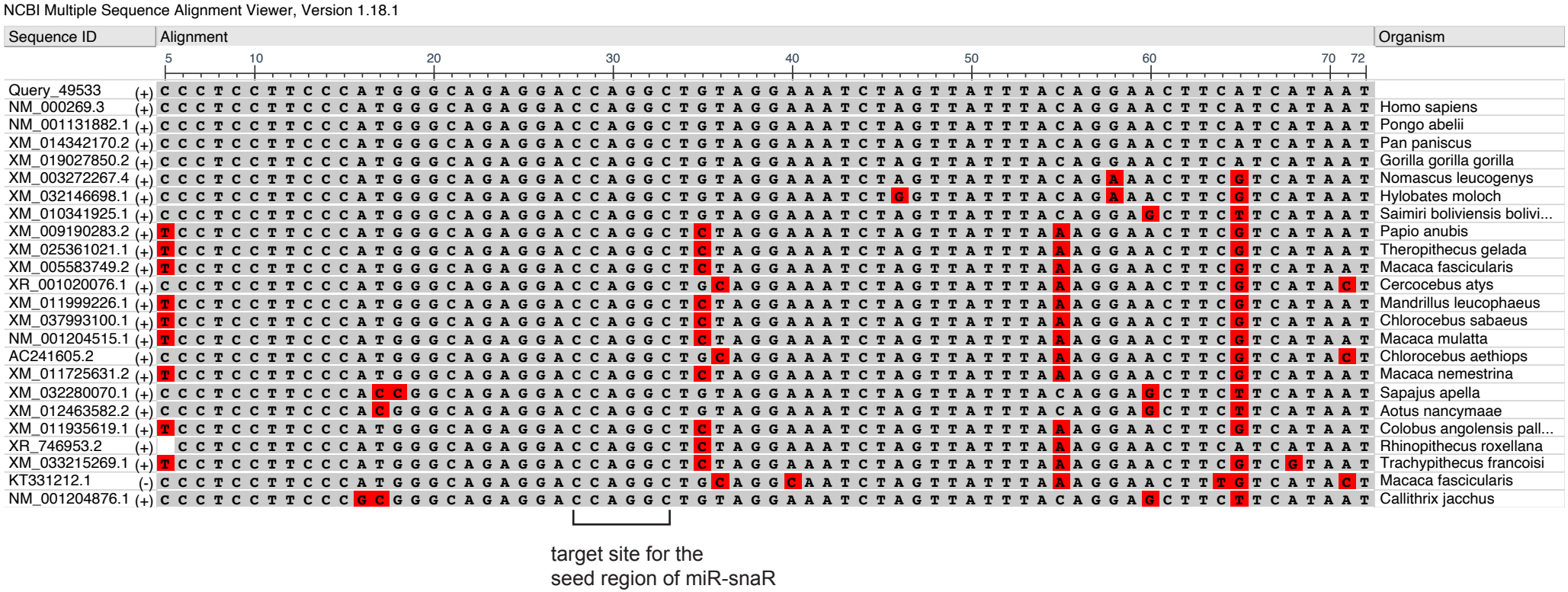
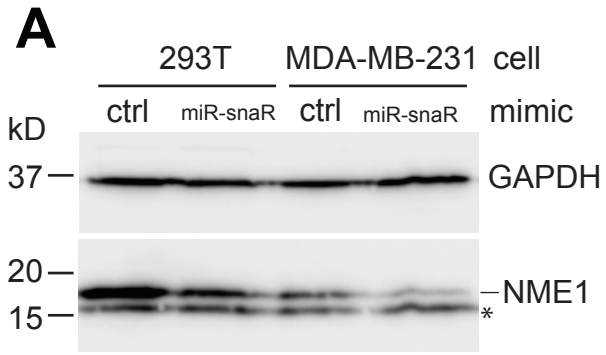
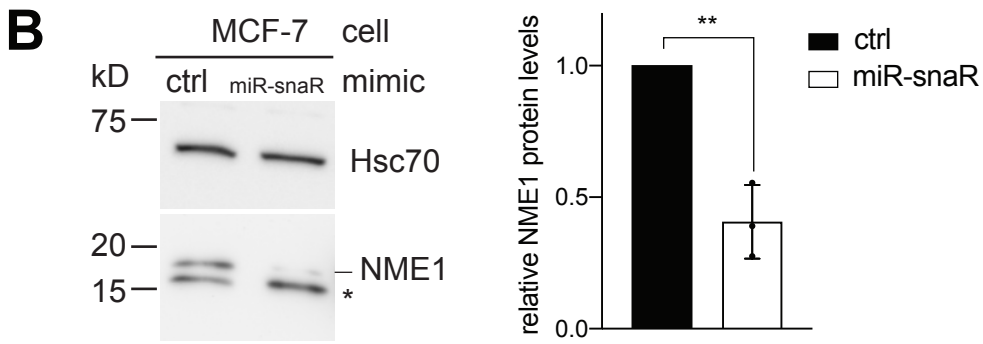
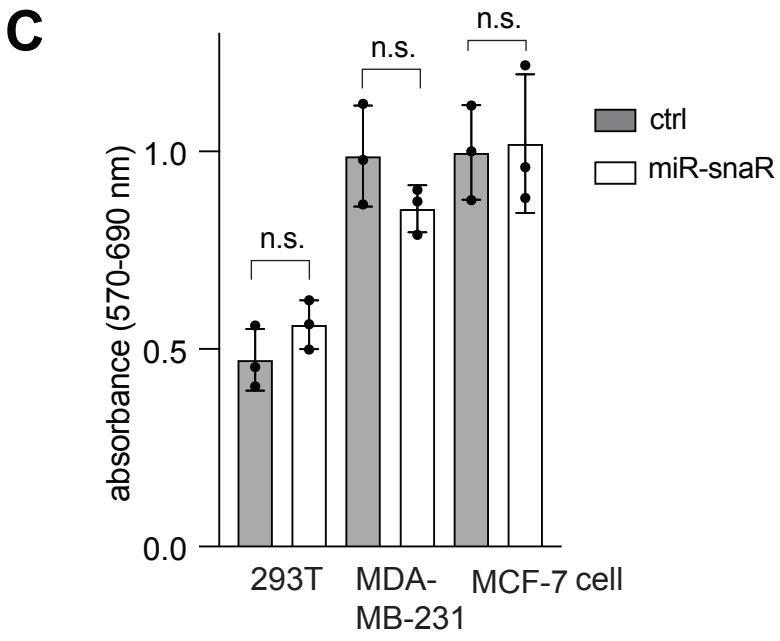

NCBI Multiple Sequence Alignment Viewer, Version 1.18.1
Sequence ID
Alignment
Organism
5
10
20
30
40
50
60
70
72
Query_49533 (+)
NM_000269.3 (+) Homo sapiens
NM_001131882.1 (+) Pongo abelii
XM_014342170.2 (+) Pan paniscus
XM_019027850.2 (+) Gorilla gorilla gorilla
XM_003272267.4 (+) Nomascus leucogenys
XM_032146698.1 (+) Hylobates moloch
XM_010341925.1 (+) Saimiri boliviensis bolivi...
XM_009190283.2 (+) Papio anubis
XM_025361021.1 (+) Theropithecus gelada
XM_005583749.2 (+) Macaca fascicularis
XR_001020076.1 (+) Cercocebus atys
XM_011999226.1 (+) Mandrillus leucophaeus
XM_037993100.1 (+) Chlorocebus sabaeus
NM_001204515.1 (+) Macaca mulatta
AC241605.2 (+) Chlorocebus aethiops
XM_011725631.2 (+) Macaca nemestrina
XM_032280070.1 (+) Sapajus apella
XM_012463582.2 (+) Aotus nancymaae
XM_011935619.1 (+) Colobus angolensis pall...
XR_746953.2 (+) Rhinopithecus roxellana
XM_033215269.1 (+) Trachypithecus francoisi
KT331212.1 (-) Macaca fascicularis
NM_001204876.1 (+) Callithrix jacchus
target site for the
seed region of miR-snaR

Figure S5


A
293T
MDA-MB-231
cell
ctrl
miR-snaR
ctrl
miR-snaR
mimic
kD
37
GAPDH
20
15
NME1
*


B
MCF-7
cell
kD
ctrl
miR-snaR
mimic
75
Hsc70
20
15
NME1
*
relative NME1 protein levels
1.0
0.5
0.0
**
ctrl
miR-snaR


C
absorbance (570-690 nm)
1.0
0.5
0.0
n.s.
n.s.
n.s.
ctrl
miR-snaR
293T
MDA-MB-231
MCF-7
cell