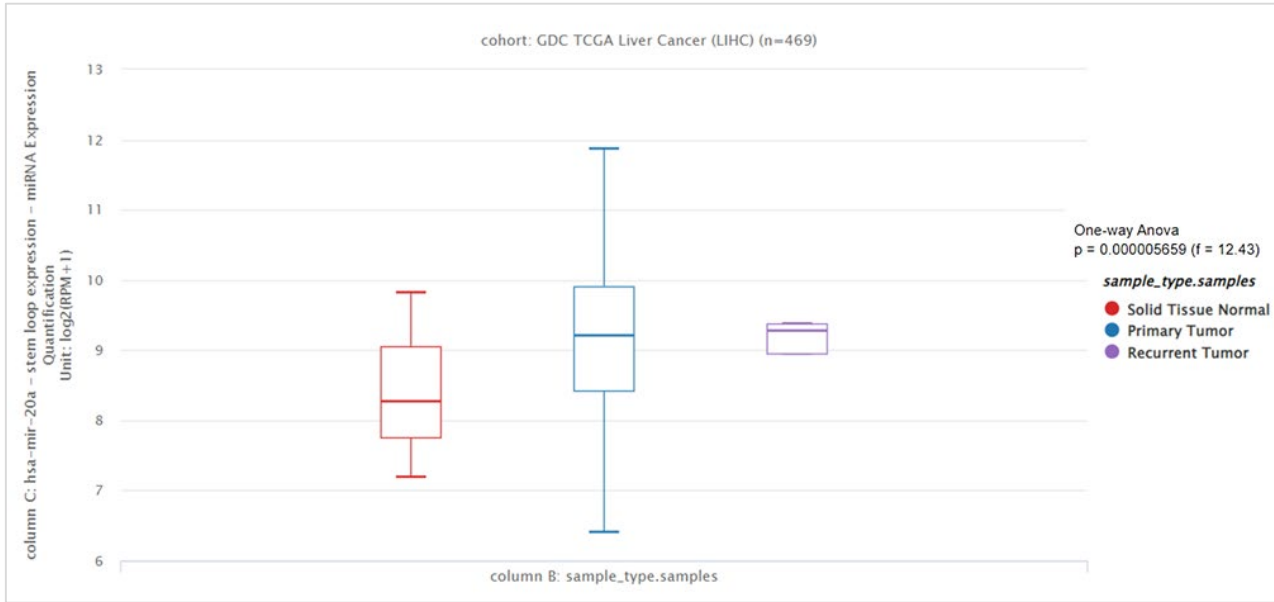


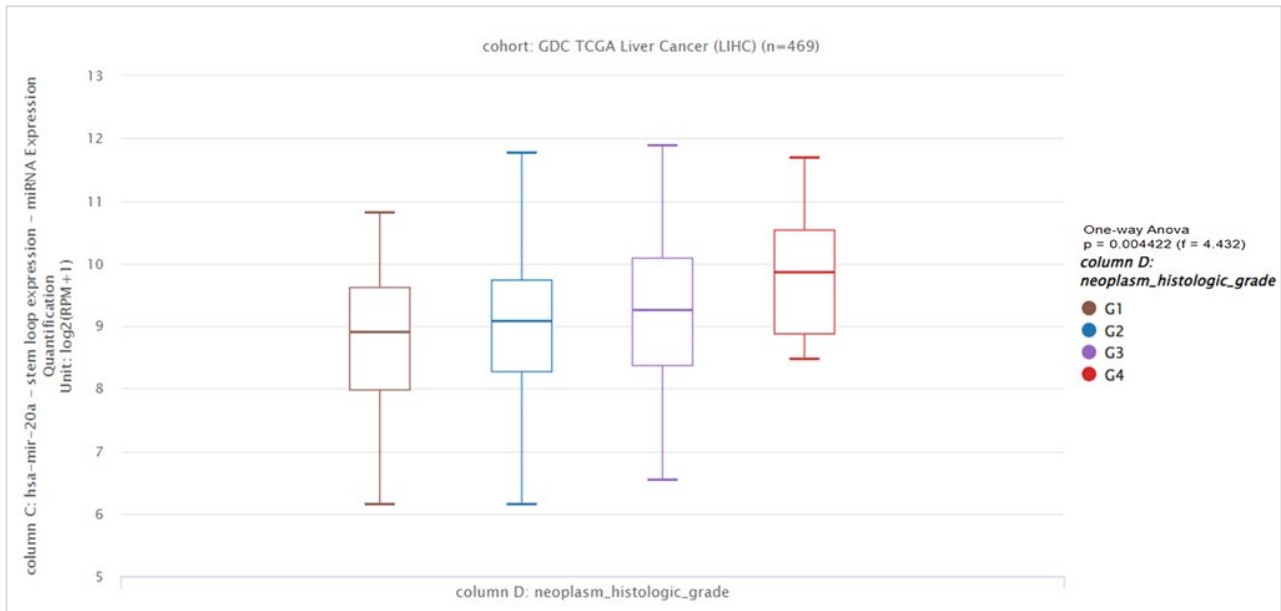
## **SUPPLEMENTARY FIGURES, LEGENDS AND TABLES**

# Supplementary Figure S1

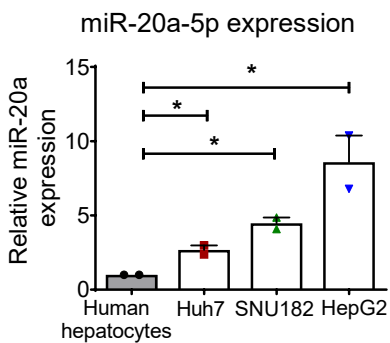
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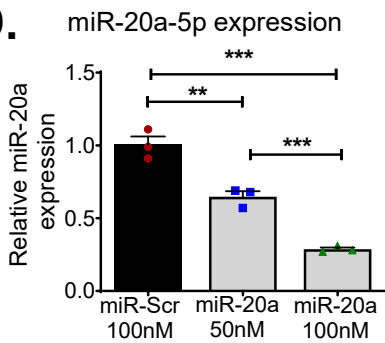
B.



C.

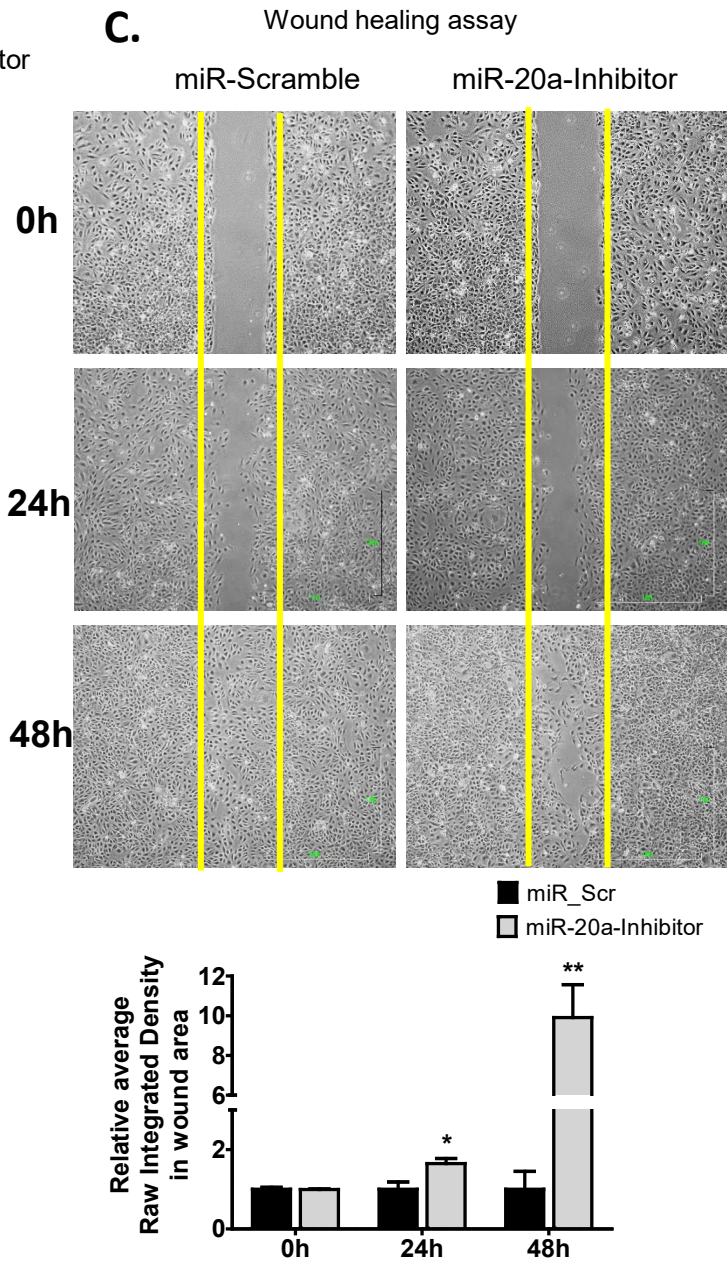
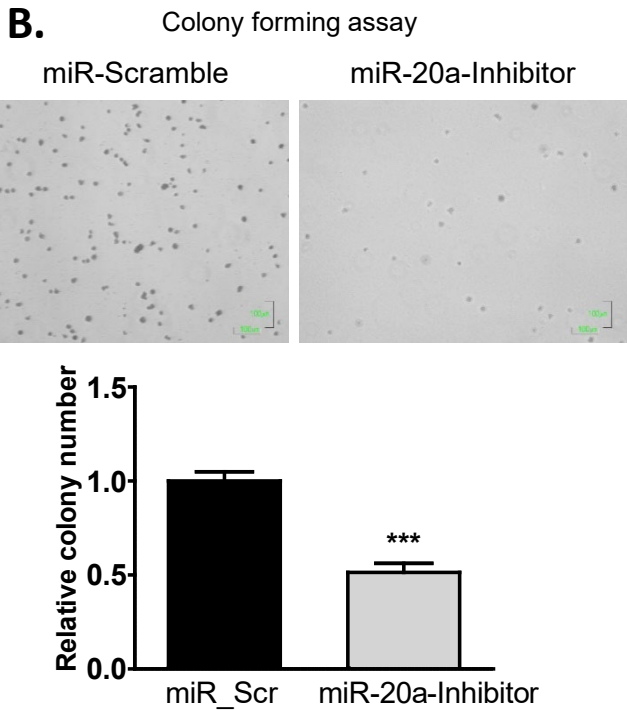
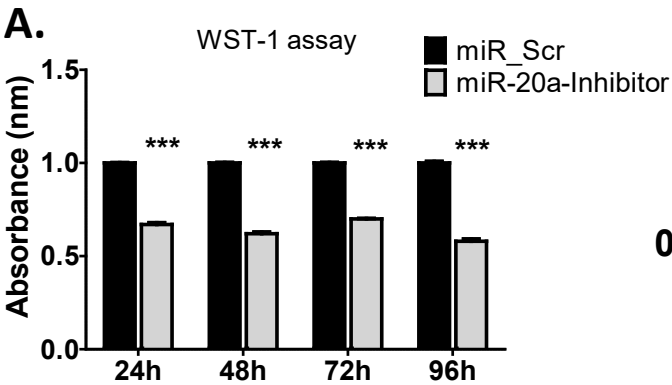


D.



**Supplementary Figure S1. MiR-20a-5p in human HCCs.** Graphs from the TCGA-LIHC dataset showing **A.** higher miR-20a-5p expression in primary tumors compared to solid tissue normal samples (One-way ANOVA,  $p=0.000005659$ ) and **B.** increasing miR-20a-5p expression with higher histological tumor grades  $G1 < G2 < G3 < G4$  (One-way ANOVA,  $p=0.004422$ ). **C.** qPCR quantification of miR-20a expression in the human HCC cell lines, Huh7, SNU182 and HepG2 relative to normal human hepatocytes. \*,  $P < 0.05$ . **D.** Determination of the optimum concentration of miR-Inhibitor for targeted knock-down of miR-20a in Huh7 cells ( $n=3$ ). \*\*,  $P < 0.008$ ; \*\*\*,  $P < 0.0004$ . The value of  $n$  equals the number of wells seeded with cells per treatment group. Unless specified, data are presented as mean  $\pm$  SEM.  $P$  values were determined by two-tailed Student's  $t$  test.

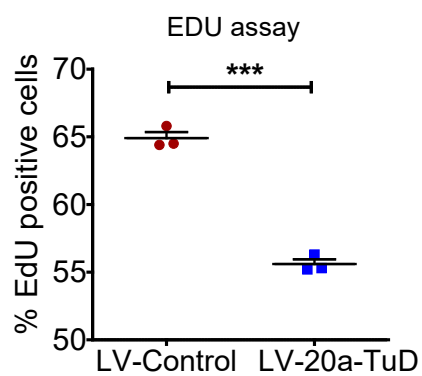
# Supplementary Figure S2



**Supplementary Figure S2. MiR-20a-5p inhibition attenuates cancer hallmarks in SNU182 cells.**

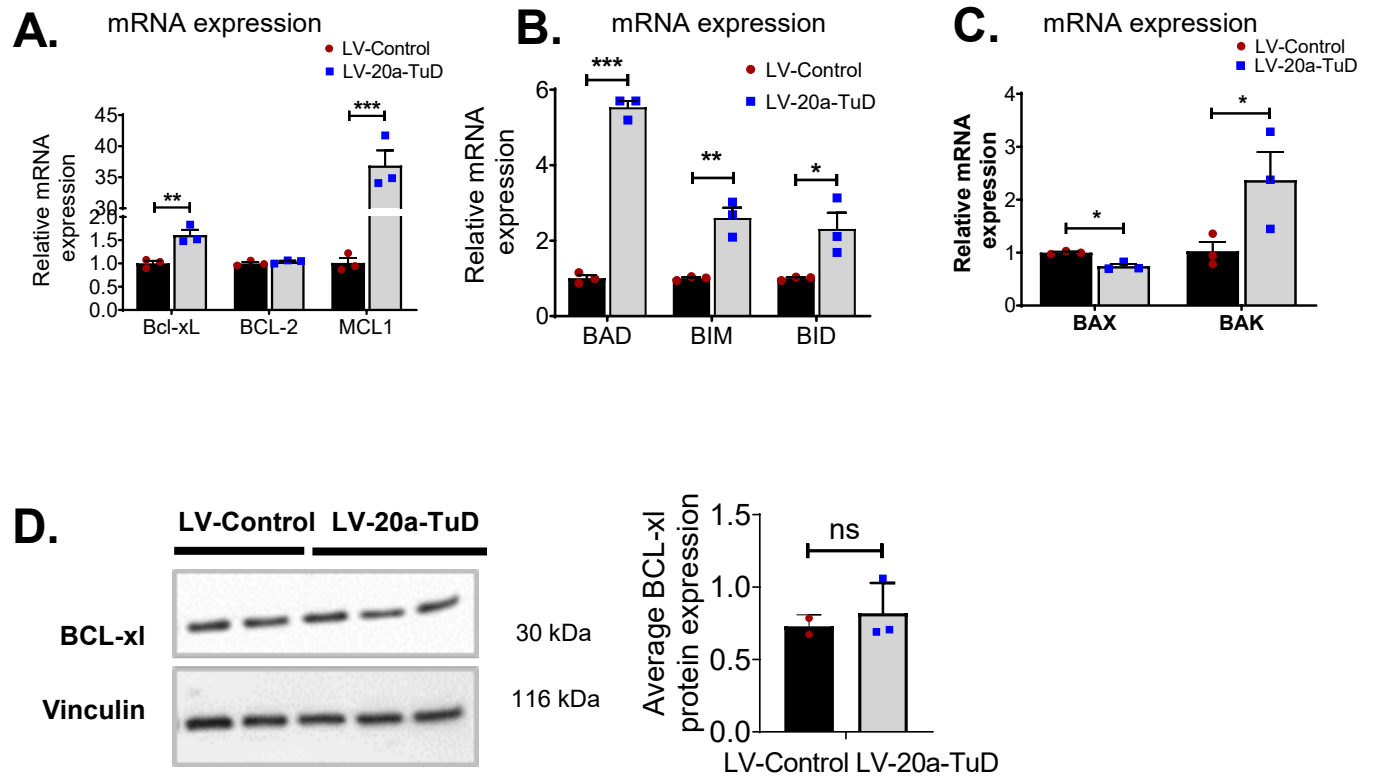
Analysing the effects of miR-20a-5p inhibition in SNU182 hepatoma cells transfected with either Scr or miR-20a-5p inhibitor. **A.** WST1 (n=10 independent biological replicates/treatment group/time point) **B.** Soft agar colony formation (n=6 independent biological replicates/treatment group) and **C.** Wound healing (n=5 independent biological replicates/treatment group) assays showed a clear inhibition of cancer hallmarks following miR-20a-5p knockdown. \*,  $P < 0.05$ ; \*\*,  $P < 0.005$ ; \*\*\*,  $P < 0.0001$ . Data are presented as mean  $\pm$  SEM.  $P$  values were determined by two-tailed Student's  $t$  test.

Supplementary Figure S3



**Supplementary Figure S3. Effect of miR-20a-5p knockdown on cell proliferation.** Click-iT EdU assay shows decreased cell proliferation in LV-20a-TuD cells compared with LV-Control cells (n=3 independent biological replicates per group). \*\*\*,  $P < 0.0001$ .

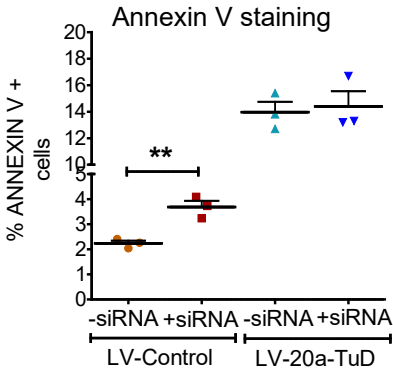
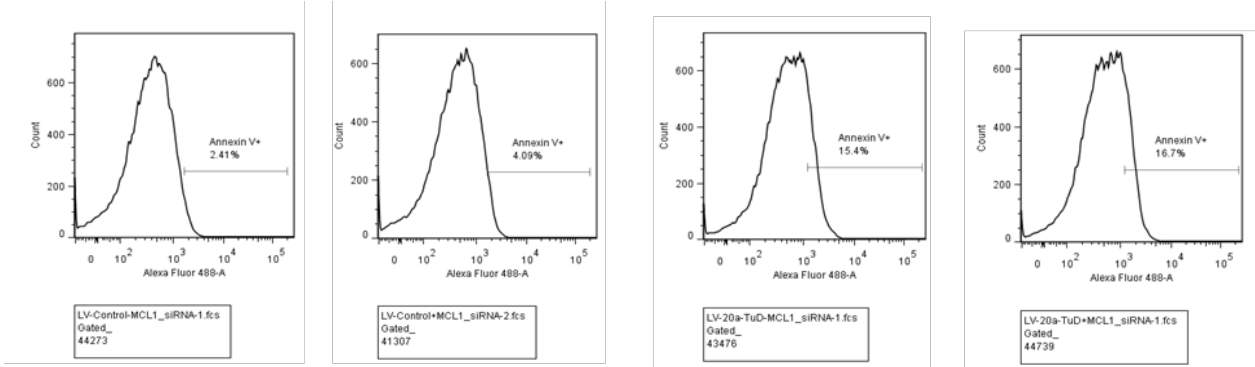
# Supplementary Figure S4



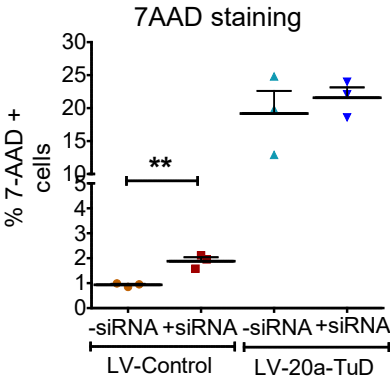
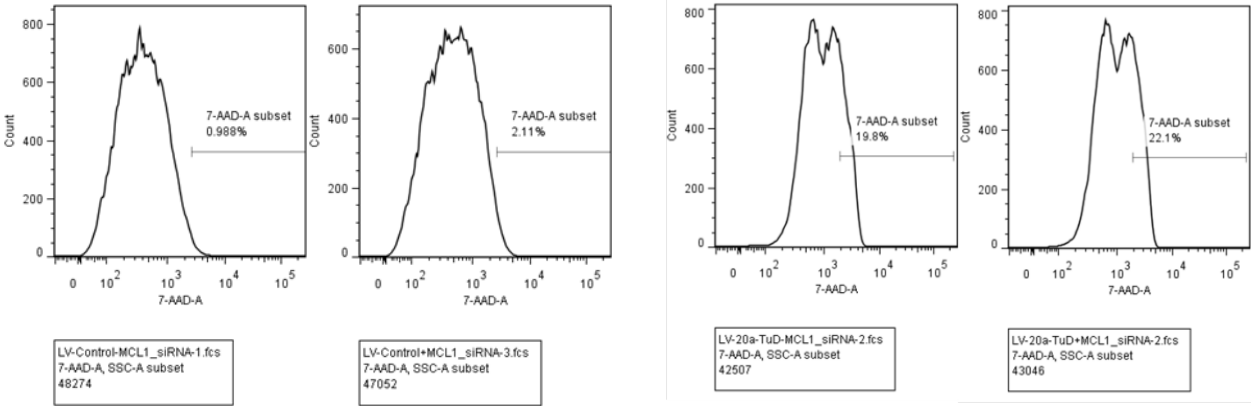
**Supplementary Figure S4. A.** qPCR quantification to determine expression of the BCL-2 family anti-apoptotic genes BCL-xL, BCL-2 and MCL1 (n=3 independent biological replicates /gene/group). \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.0001$ . **B.** Determination of the mRNA expression of key BCL-2 pro-apoptotic genes BAD, BIM and BID via qPCR. (n=3 independent biological replicates /gene/group).\*,  $P < 0.01$ ; \*\*,  $P < 0.001$ ; \*\*\*,  $P < 0.0001$ . **C.** qPCR to determine mRNA expression of apoptosis effectors BAX and BAK (n=3 independent biological replicates /gene/group). \*,  $P < 0.05$ . LV-20a-TuD cells are compared to LV-Control cells. **D.** Western blot shows no significant increase in BCL-xL protein expression in LV-20a-TuD cells compared to LV-Control cells (LV-Control: n=2; LV-20a-TuD n=3 independent biological replicates). Data are presented as mean  $\pm$  SEM.  $P$  values were determined by two-tailed Student's  $t$  test.

Supplementary Figure S5

A. Annexin V

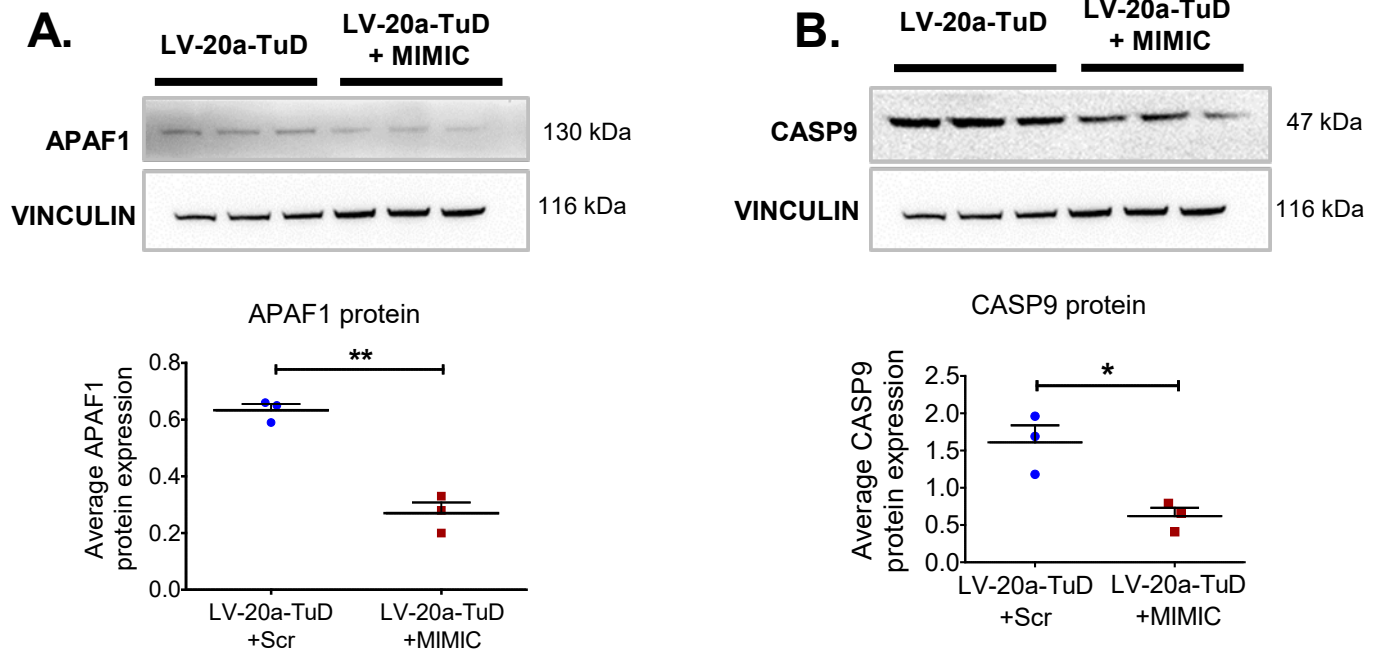


B. 7-AAD



**Supplementary Figure S5.** Representative histograms and graphs from flow cytometry analyses showing **A.** ANNEXIN V positive and **B.** 7-AAD positive LV-Control and LV-20a-TuD cells transfected with MCL1 siRNA and siRNA-Negative control (n=3). The value of n equals the number of independent biological replicates per treatment group. Data are presented as mean  $\pm$  SEM. *P* values were determined by two-tailed Student's *t* test. ANNEXIN V: \*\*, *P* = 0.005. 7-AAD: \*\*, *P* = 0.004.

## Supplementary Figure S6



**Supplementary Figure S6.** Western blots show significant downregulation of protein expression of **A.** APAF1 and **B.** Caspase 9 (CASP9) in LV-20a-TuD-MIMIC treated cells compared to scramble treated LV-20a-TuD cells (n=3). The value of n equals the number of independent biological replicates per treatment group. Data are presented as mean  $\pm$  SEM. *P* values were determined by two-tailed Student's *t* test. APAF1: \*\*, *P* < 0.002. CASP9: \*, *P* < 0.02.

**Supplementary Table S1****A. List of qPCR SYBR Green Primers**

| <b>SYBR Green Primers</b> | <b>Forward Primer</b>    | <b>Reverse Primer</b>   |
|---------------------------|--------------------------|-------------------------|
| hsa/mmu $\beta$ -actin    | GATCAAGATCATTGCTCCTCCT   | TGATCCACATCTGCTGGAAG    |
| hsa-BIM                   | CAGTGCAATGGCTTCCATG      | AATACCCTCCTTGCATAGTAAGC |
| hsa-BAX                   | CGAGTGGCAGCTGACATG       | GTGCACAGGGCCTTGAG       |
| hsa-BAD                   | GGACTCCTTTAAGAAGGGACTTC  | CCACCAGGACTGGAAGACTC    |
| hsa-BID                   | CGGATTCTGTCGCCACTG       | ACCGTTGTTGACCTCACAGTC   |
| hsa-BCL-XL                | GTAGACAAGGAGATGCAGGTATTG | TCCACAAAAGTATCCCAGCC    |
| hsa-MCL1                  | GCCTTCCAAGGCATGCT        | CAGTTTGTTACGCCGTCG      |
| hsa-BCL2                  | GTCAACCGGGAGATGTCG       | TCCACAAAGGCATCCCAG      |
| hsa-BAK                   | GCTTCTGAGGAGCAGGTAGC     | CCATGGTGCTGCTAGGTTG     |
| hsa-CYCS                  | TCTCCATGGTCTCTTTGGGC     | ATCCTCTCCCCAGATGATGC    |
| mmu-CYCS                  | AGGCTGCTGGATTCTCTTACAC   | GTTCCAGGGATGTACTTTTTGG  |
| hsa-Casp3                 | TGCATACTCCACAGCACCTG     | TCAAGCTTGTCGGCATACTG    |
| hsa-Casp7                 | TGTGCTTCTTATGTTACCCAGAT  | CTTGACAAACCAGGAGCCTC    |
| AAV-TTR-Titration         | TCAGCTTGGCAGGGATCAG      | GACGGCTTCTCCTGGTGAAG    |
| U6-20a-TuD-For            | GCATATACGATACAAGGCTG     |                         |

**B. qPCR TaqMan probes**

| <b>TaqMan Probe</b>     | <b>Company</b>          | <b>Catalogue Number</b> | <b>Assay ID</b> |
|-------------------------|-------------------------|-------------------------|-----------------|
| U6 snRNA TaqMan control | ThermoFisher Scientific | 4440887                 | 001093          |
| hsa/mmu-miR-20a         | ThermoFisher Scientific | 4427975                 | 000580          |

## Supplementary Table S2

### Organoid culture medium

| Stock concentration                  | Final concentration | Volume |
|--------------------------------------|---------------------|--------|
| AdDMEM/F12                           |                     | 50mL   |
| HEPES 1M                             | 10mM                | 0.5mL  |
| GlutaMax 100X                        | 1X                  | 0.5mL  |
| Penicillin-Streptomycin100X          | 1X                  | 0.5mL  |
| RSPO 50µg/mL                         | 150ng/mL            | 150µl  |
| B27 50X                              | 1X                  | 1mL    |
| N-acetylcysteine (Sigma)<br>500mM/mL | 1.25mM/mL           | 125µl  |
| Nicotinamide (Sigma)1M/mL            | 10mM/mL             | 0.5mL  |
| gastrin (Sigma) 500µg/mL             | 20ng/mL             | 2µl    |
| CHIR99021 10nM/mL                    | 3µM/mL              | 15µl   |
| EGF (Peprotech) 100µg/mL             | 5ng/mL              | 5µl    |
| HGF (Peprotech) 100µg/mL             | 5ng/mL              | 5µl    |
| FGF7 (Peprotech) 100µg/mL            | 5ng/mL              | 5µl    |
| FGF10 (Peprotech)<br>100µg/mL        | 5ng/mL              | 5µl    |
| A83-01 5mM/mL                        | 1µM/mL              | 10µl   |
| Y27632 3mg/mL(10mM/mL)               | 3µg/mL(10µM/mL)     | 50µl   |