

Supplemental information

**Systematic identification of clinically
relevant miRNAs for potential miRNA-based
therapy in lung adenocarcinoma**

Shu-Hsuan Liu, Kai-Wen Hsu, Yo-Liang Lai, Yu-Feng Lin, Fang-Hsin Chen, Pei-Hwa Peng, Li-Jie Lin, Heng-Hsiung Wu, Chia-Yang Li, Shu-Chi Wang, Min-Zu Wu, Yuh-Pyng Sher, and Wei-Chung Cheng

Supplemental Figures

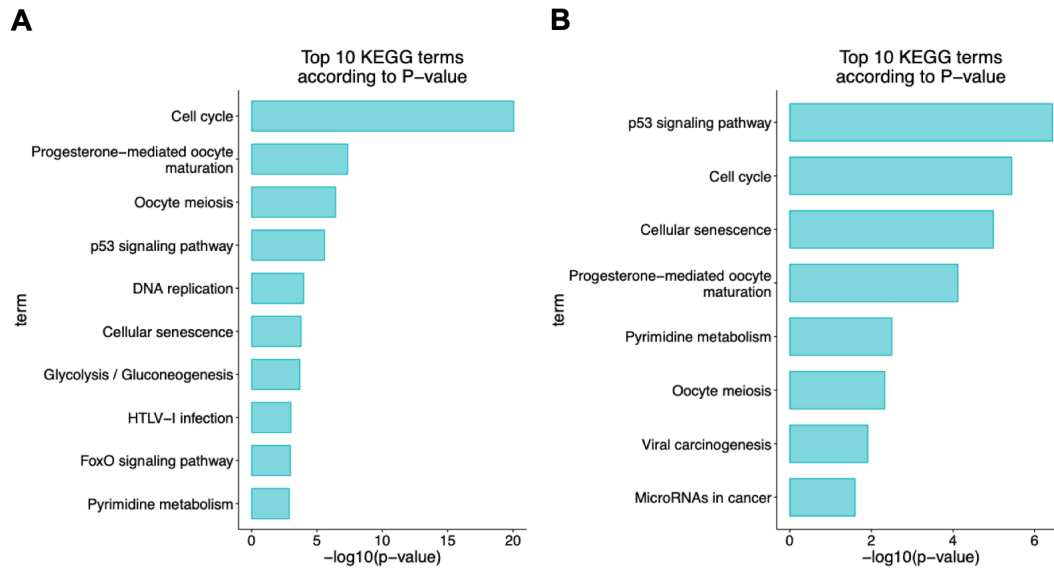


Figure S1. Functional enrichment of target genes regulated by miRNA candidates. (A) There were 349 targets that were differentially expressed and showed survival significance, and functional annotation of these targets was performed. (B) Functional annotation of the target genes regulated by the final three miRNA candidates also involved in similar functions.

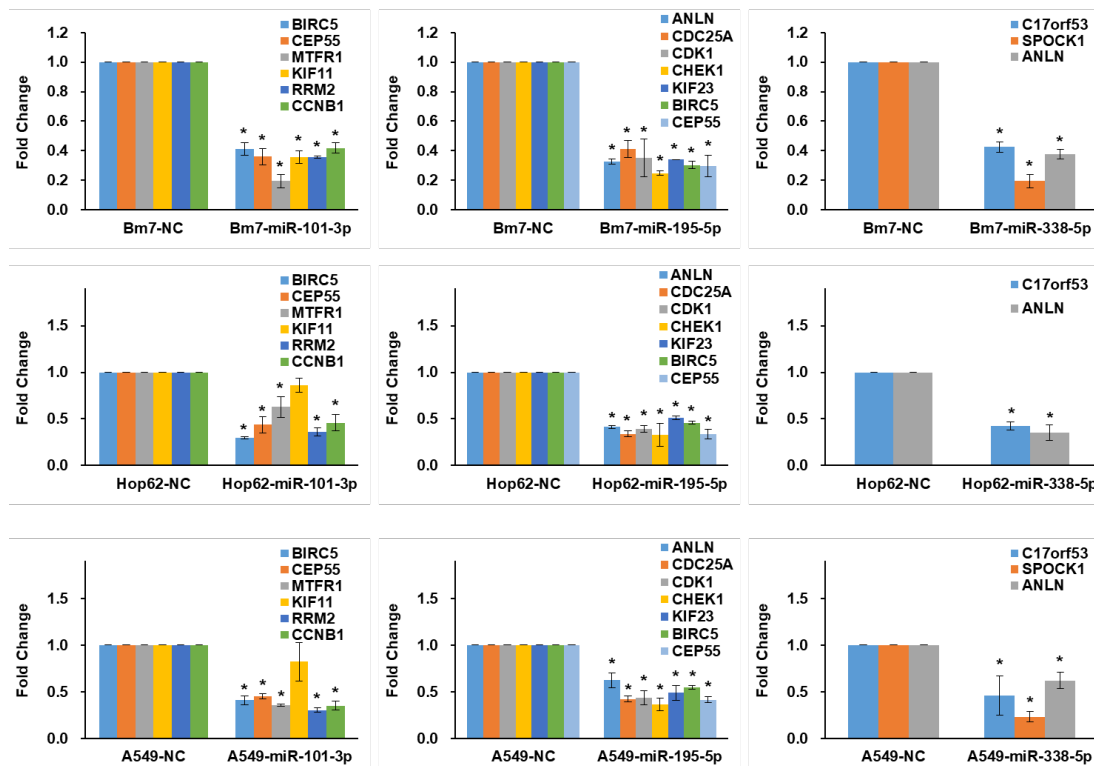


Figure S2. Expression analysis of miRNA target genes using quantitative real-time PCR followed by

transfection with miR-195-5p, miR-101-3p, and miR-338-5p mimics. The results of quantitative real-time PCR analysis revealed that the expression levels of the target genes of miR-195-5p, miR-101-3p, and miR-338-5p were decreased in A549, Bm7, and Hop62 cells followed by transient transfection with the respective miRNA mimics. A scrambled oligonucleotide was used as the negative control (NC). All the cell lines were individually transfected with the miRNA mimics at a final concentration of 20 nM. The mean values obtained from at least three independent experiments are depicted here. The error bars depict the standard error of mean. The asterisk (*) indicates the statistical significance ($P < 0.05$) between the experimental and control groups.

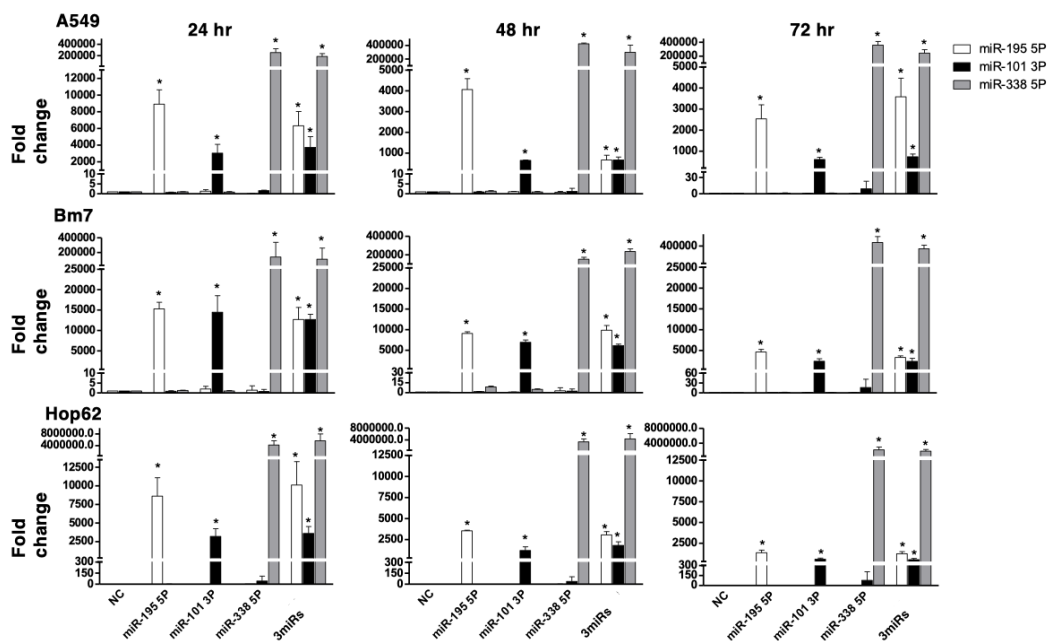


Figure S3. Detection of miRNAs by quantitative real-time PCR following transfection with miR-195-5p, miR-101-3p, and miR-338-5p mimics. The results of quantitative real-time PCR analysis for miRNA detection revealed that the expression levels of miR-195-5p, miR-101-3p, and miR-338-5p increased in A549, Bm7, and Hop62 cells following transient transfection with the miRNA mimics at 24, 48, and 72 h. A scrambled oligonucleotide was used as negative control (NC). All the cells were individually transfected with the miRNA mimics at a final concentration of 20 nM. In the “combined” setup, the cells were transfected with all three of the miRNA mimics at one-third concentrations. The mean values obtained from at least three independent experiments are depicted. The error bars depict the standard error of each mean. The asterisks (*) indicate statistically significant differences ($P < 0.05$) between the experimental and control groups.

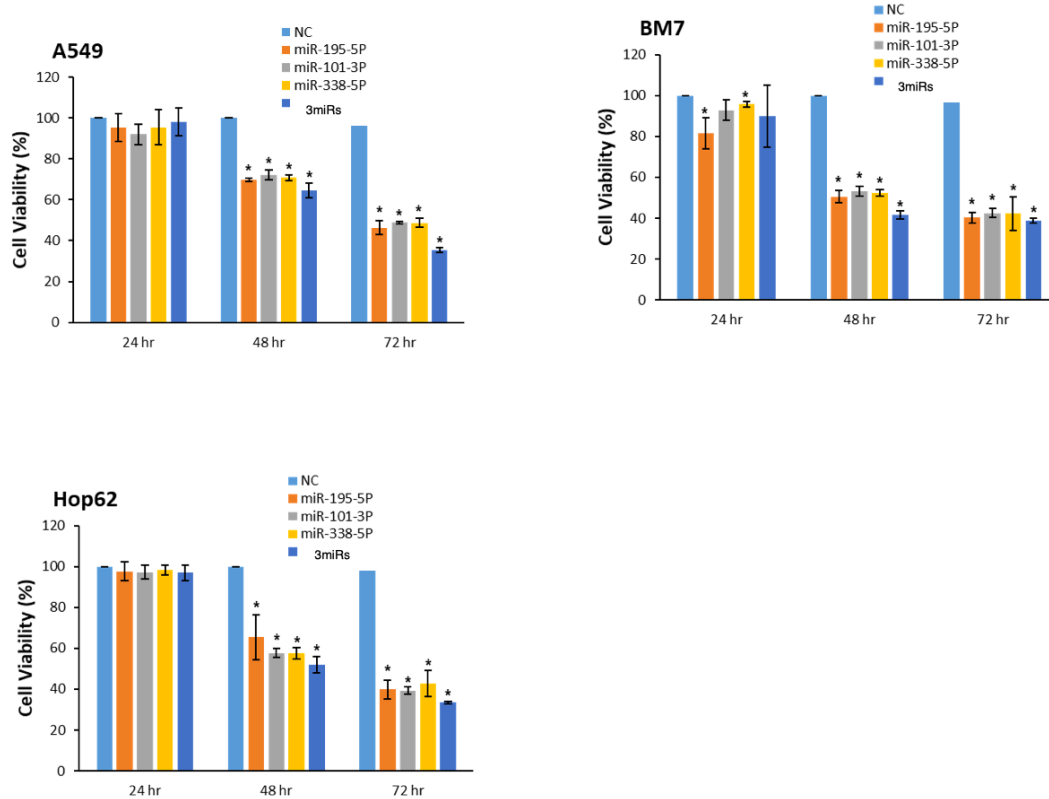


Figure S4. MTT assay of overexpressed miR-195-5p, miR-101-3p, or miR-338-5p in A549, Bm7, and Hop62 cells. A549, Bm7, and Hop62 cells that had been transfected with miR-195-5p, miR-101-3p, or miR-338-5p mimics were seeded onto 24-well plates and incubated for 24, 48, or 72 h. MTT assays showed that the overexpression of the miRNA mimics decreased cell viability in all three lung cancer cell lines. 3miRs is denoted as the combination of three miRNAs (miR-195-5p, miR-101-3p, and miR-338-5p).

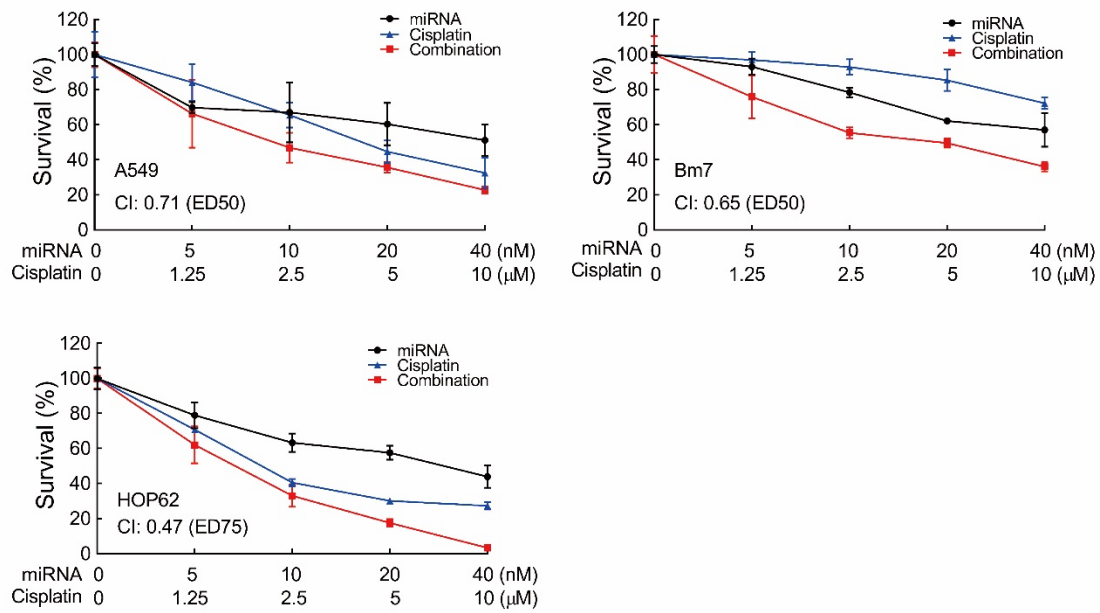


Figure S5. Synergistic therapeutic effects of miRs and cisplatin in A549, Bm7, and HOP62 cells by MTT assay. CI, combination index. In HOP62, CI: 0.96 (ED50).

Supplemental Tables

Table S1. miRNA-target interactions with the evidence level.

miRNA	gene	Evidence level
hsa-miR-101-3p	BIRC5	miRTarbase
	CCNB1	Prediction tools ≥ 6
	KIF11	Prediction tools ≥ 6
	RRM2	miRTarbase
	MTFR1	Prediction tools ≥ 6
	CEP55	Prediction tools ≥ 6
hsa-miR-195-5p	BIRC5	miRTarbase
	CDK1	miRTarbase
	CDC25A	miRTarbase
	CHEK1	miRTarbase
	KIF23	miRTarbase
	ANLN	Prediction tools ≥ 6
	CEP55	miRTarbase
hsa-miR-338-5p	SPOCK1	Prediction tools ≥ 6
	ANLN	Prediction tools ≥ 6
	C17orf53	Prediction tools ≥ 6

Table S2. Sequence of the oligonucleotides for real-time PCR

Target	Sequence(5'→3')
ANLN	F: ATCTTGCTGCAACTATTTGCTCC
	R: TCCTGCTTAACACTGCTGCTA
BIRC5	F: AGGACCACCGCATCTCTACAT
	R: AAGTCTGGCTCGTTCTCAGTG
C17orf53	F: CATCCACAAAGCGGGTATCAT
	R: TGAGTGGAACTGTTAAGGCA
CCNB1	F: AATAAGGCGAAGATCAACATGGC
	R: TTTGTTACCAATGTCCCAAGAG
CDC25A	F: TTCCTCTTTTTACACCCAGTCA
	R: TCGGTTGTCAAGGTTTGTAGTTC
CDK1	F: GGATGTGCTTATGCAGGATTCC
	R: CATGTACTGACCAGGAGGGATAG
CEP55	F: CTGGAAGAGACAACGAGAGAAGG
	R: CAAGTTCAGCAATTCGTGAGGT
CHEK1	F: CCAGATGCTCAGAGATTCTTCCA
	R: TGTTCAACAAACGCTCACGATTA

KIF11	F: TCCCTGGCTGGTATAATTCCA
	R: GTTACGGGGATCATCAAACATCT
KIF23	F: TACCATTGAATCGTGAGTCCA
	R: CTCTGGTCCGGTTAGTTCTTTC
MTFR1	F: ATGTTGGATGGGTAGCCAAAG
	R: TTCGAGAGCGCAAATCTTCTG
RRM2	F: GTGGAGCGATTTAGCCAAGAA
	R: CACAAGGCATCGTTTCAATGG
SPOCK1	F: ACCCCTGCCTGAAGGTAAAAT
	R: GGCTTGCACTGACCAAATTC
18s	F: GCGGGCGTTATTCCCATGA
	R: GAGGTTTCCCGTGTTGAG