

FIGURE S1. Huh7, Bel-7402, HepG2 and SMMC-7721 cells were treated for 24 hours with the indicated 50ng/ml of TRAIL, and cell viability was determined by CCK-8 assay.

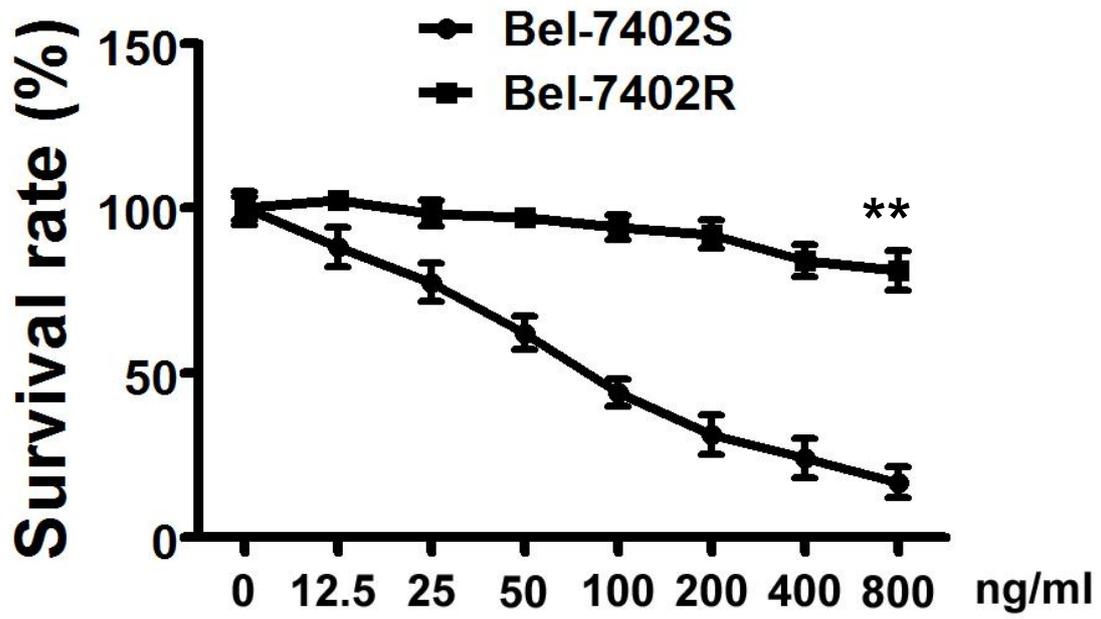


FIGURE S2. Bel-7402S and Bel-7402R cells were treated for 24 hours with the indicated concentrations of TRAIL, and cell viability was determined by CCK-8 assay.

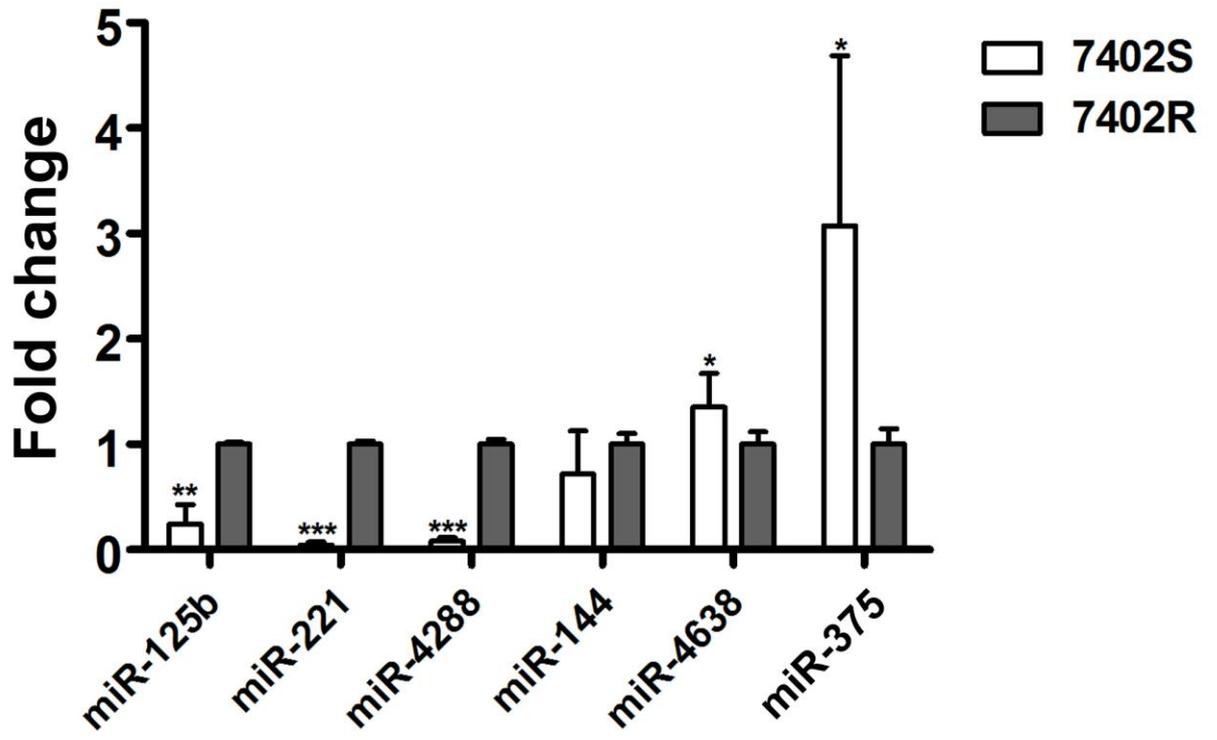


FIGURE S3. qRT-PCR analysis of six altered miRNAs in Bel-7402S and Bel-7402R cells.

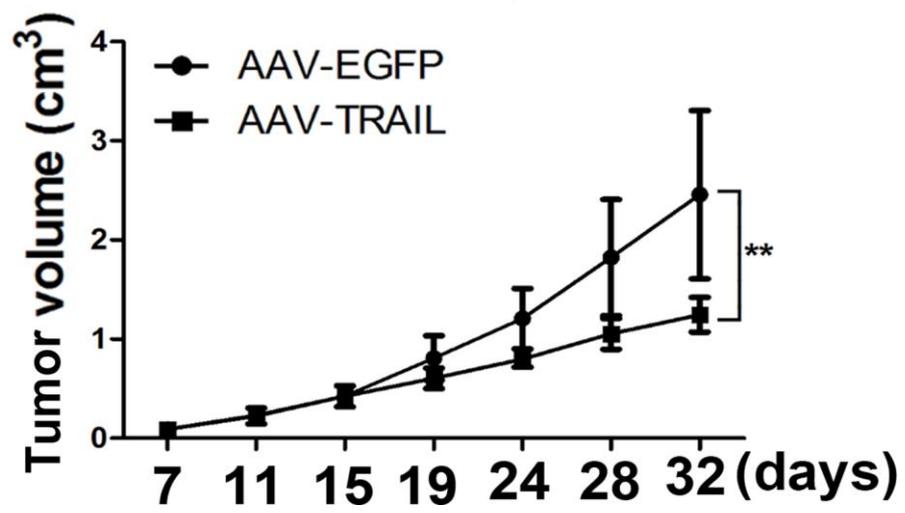


FIGURE S4. Bel-7402 tumor volume in different time points after AAV-EGFP and AAV-TRAIL injection.

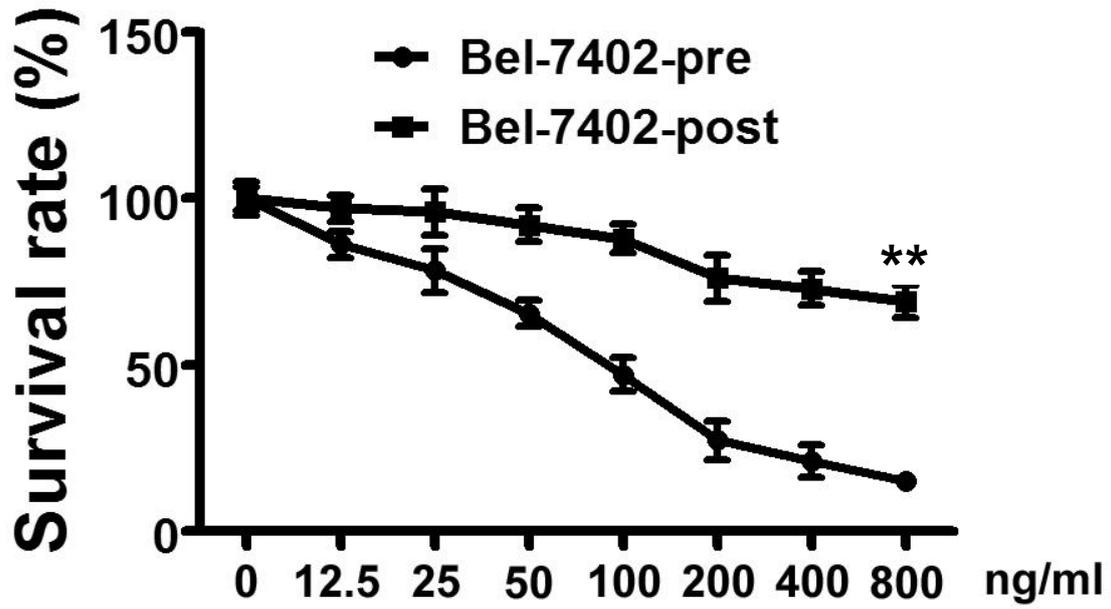


FIGURE S5. Bel-7402-pre and Bel-7402-post cells were treated for 24 hours with the indicated concentrations of TRAIL, and cell viability was determined by CCK-8 assay.

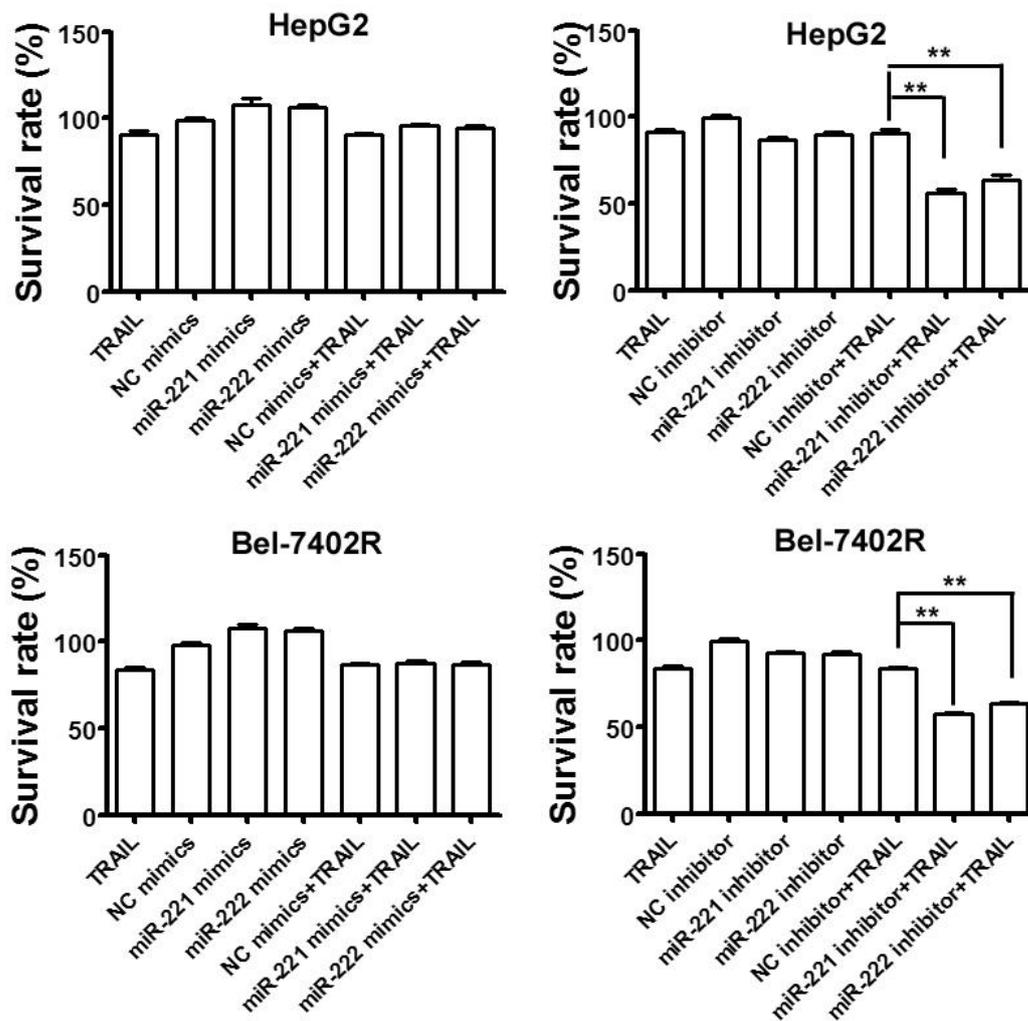


FIGURE S6. MiR-221/222 knockdown increases TRAIL sensitivity in TRAIL-resistant liver cancer cells. CCK-8 assay was used to detect the cell viability of HepG2 and Bel-7402R cells to 500ng/ml TRAIL 24 hours after transfected with miR-221/222 mimics or inhibitors.

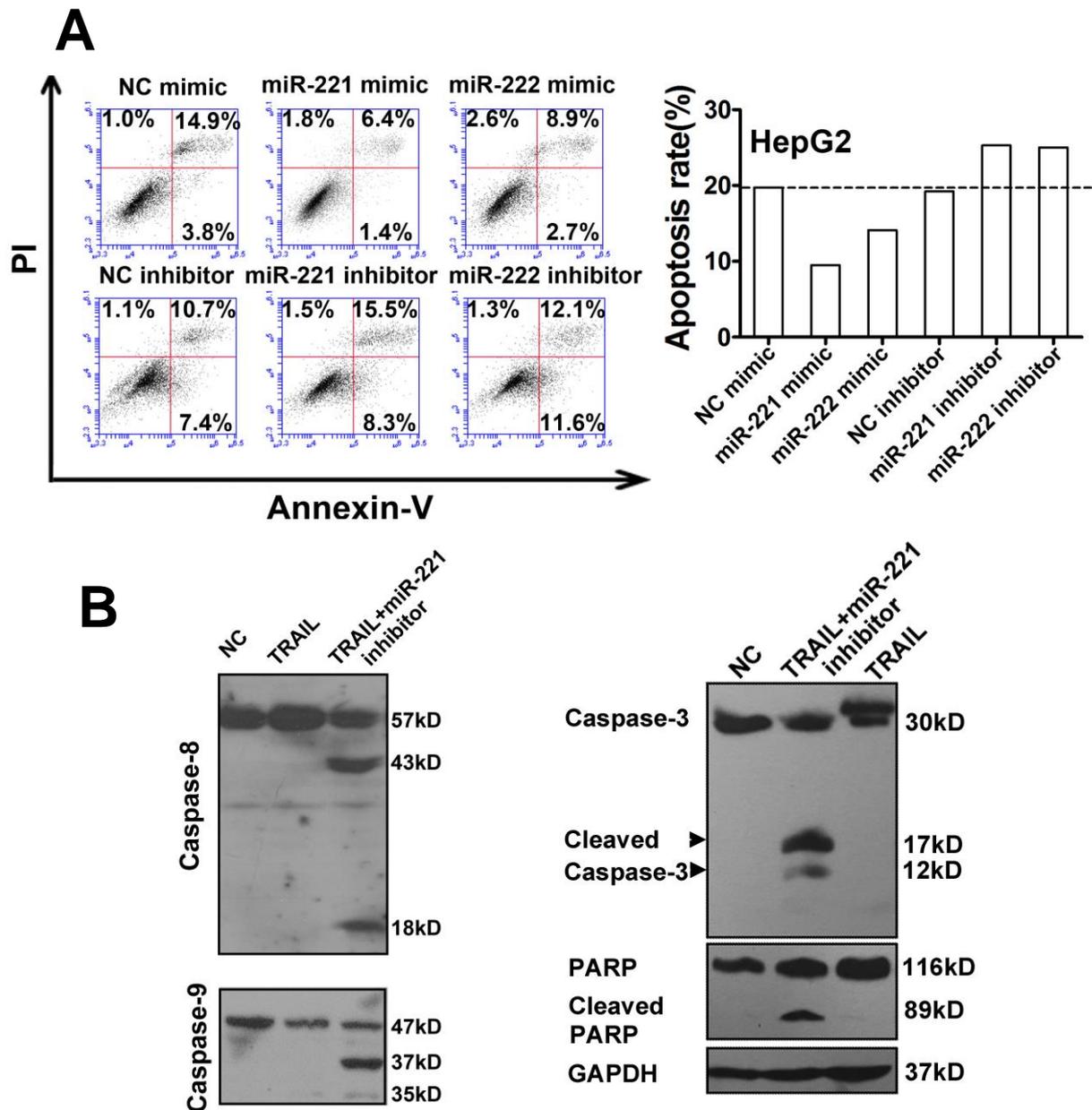


FIGURE S7. MiR-221/222 knockdown increases TRAIL sensitivity in HepG2 cells. (A) Flow cytometry assay was used to detect the HepG2 cell apoptosis rate stimulated with 500ng/ml TRAIL 24 hours after transfected with miR-221/222 mimics or inhibitors. (B) Western blot assay was used to the pre form and cleavage Caspase-9, Caspase-8, Caspase-3 and PARP in HepG2 cells stimulated with 500ng/ml TRAIL 24 hours after transfected with miR-221/222 mimics or inhibitors.

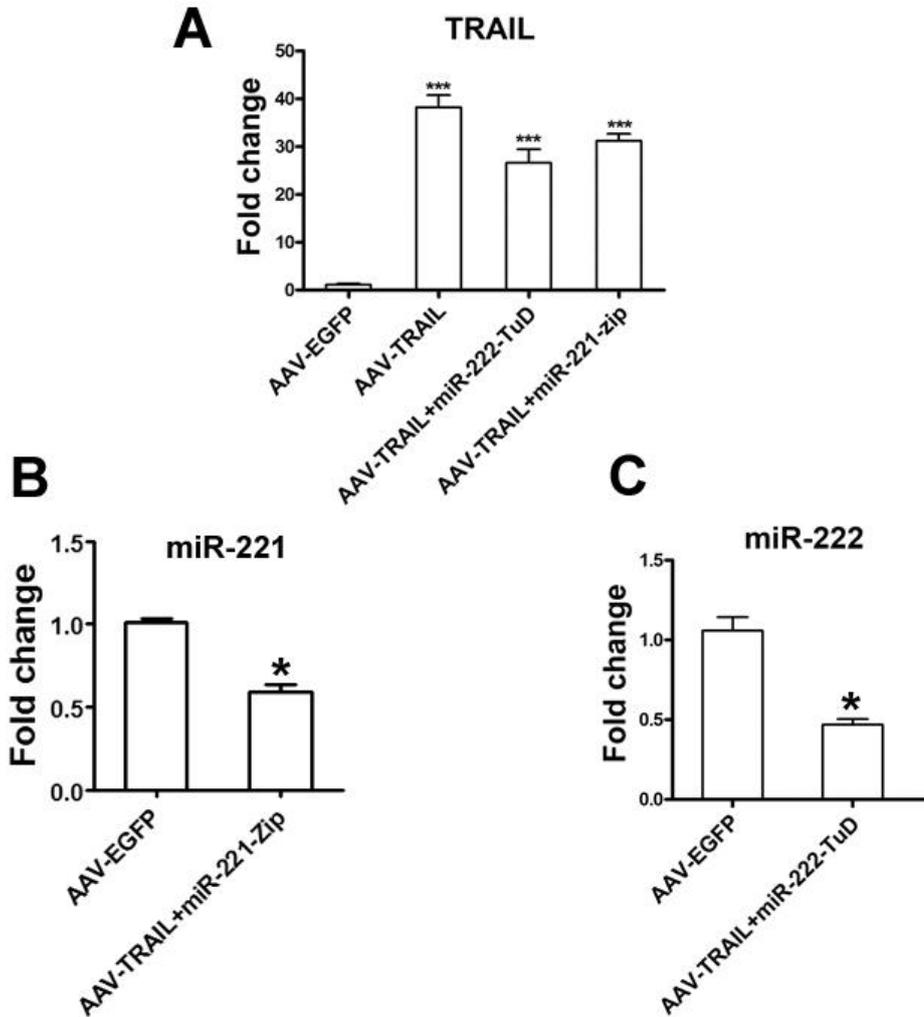


FIGURE S8. AAV-TRAIL-miR-221-Zip and AAV-TRAIL-miR-222-TuD viruses inhibit miR-222 and miR-221 expression *in vitro*. (A) qRT-PCR analysis of the relative expression of TRAIL in HEK 293T cells after infection with AAV-EGFP, AAV-TRAIL, AAV-TRAIL-miR-222-TuD and AAV-TRAIL-miR-221-Zip. (B) qRT-PCR analysis of the relative expression of miR-221 in HEK 293T cells after infection with AAV-EGFP and AAV-TRAIL-miR-221-TuD. (C) qRT-PCR analysis of the relative expression of miR-222 in HEK 293T cells after infection with AAV-EGFP and AAV-TRAIL-miR-221-Zip. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

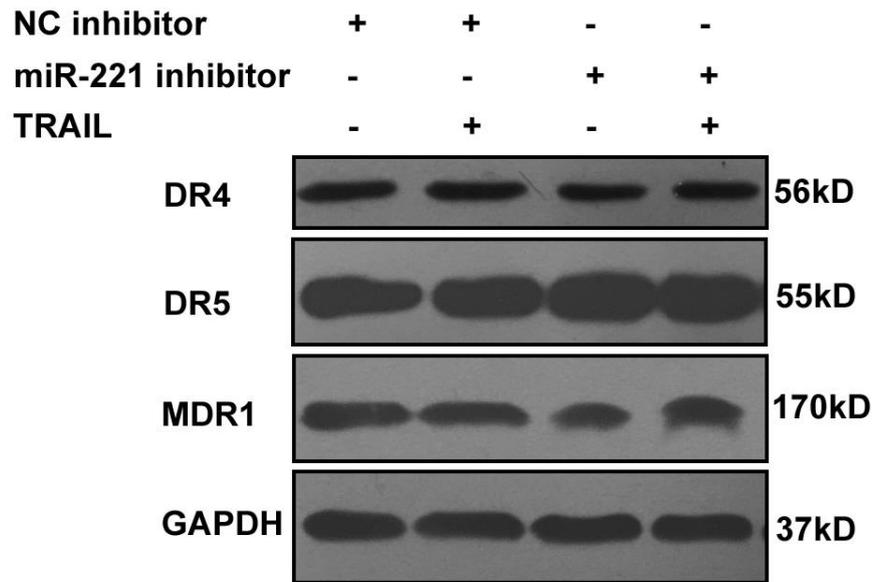
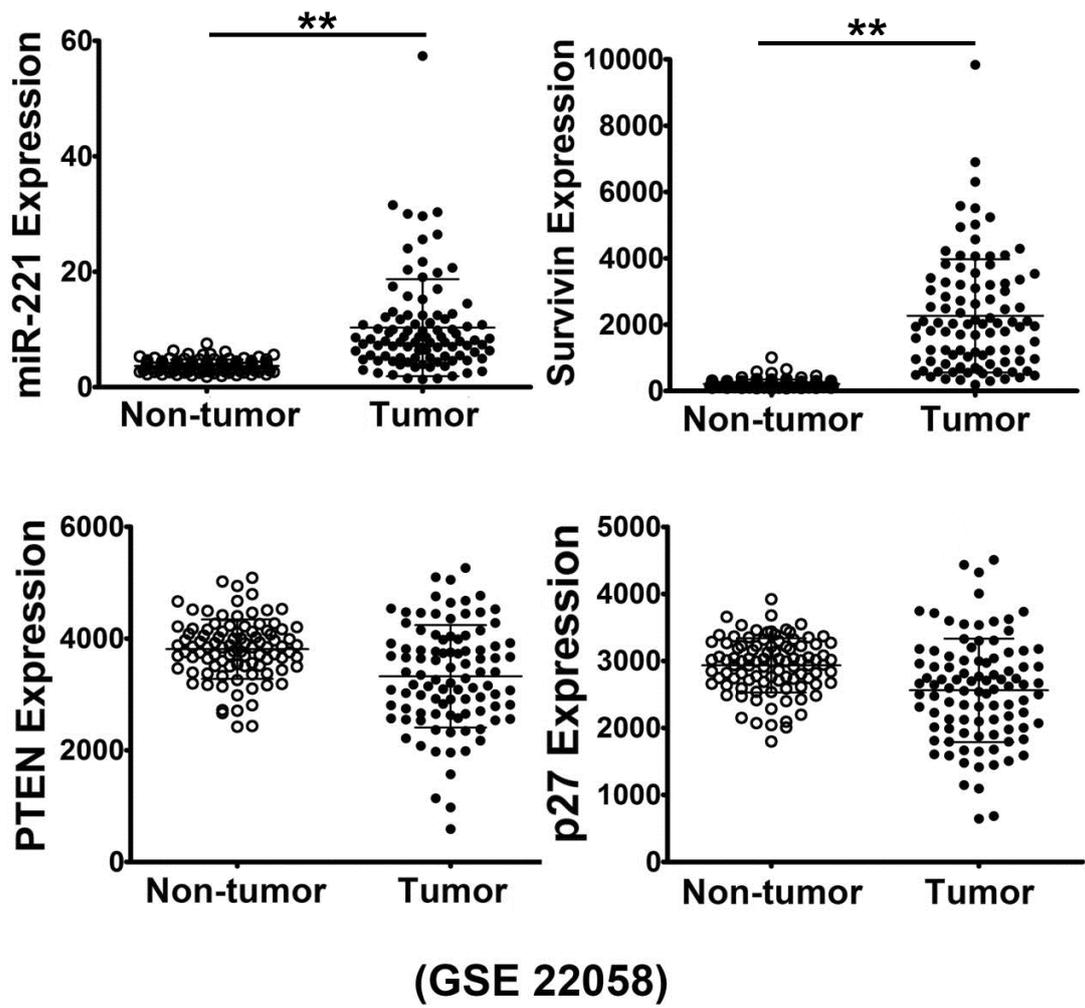


FIGURE S9. Western blot analysis of the expression of DR4, DR5 and MDR1 in HepG2 cells after TRAIL stimulation and/or transfection with a miR-221 inhibitor.



(GSE 22058)

FIGURE S10. Expression analysis of miR-221, Survivin, PTEN and p27 in liver cancer patients from GSE22058 database. ** $p < 0.01$

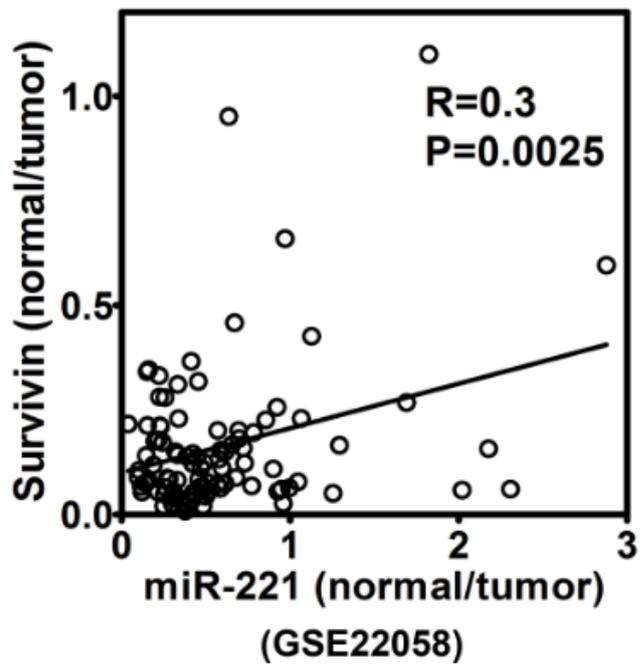


FIGURE S11. The correlation analysis of miR-221 and Survivin expression in liver cancer patients from GSE22058 database.

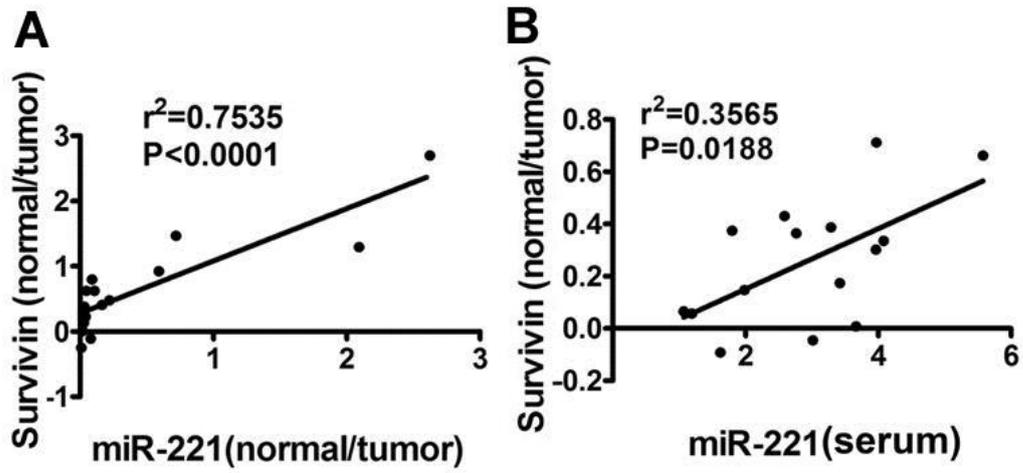


FIGURE S12. The correlation analysis of miR-221 and Survivin in tumors of the 15 liver cancer patients or in serum.

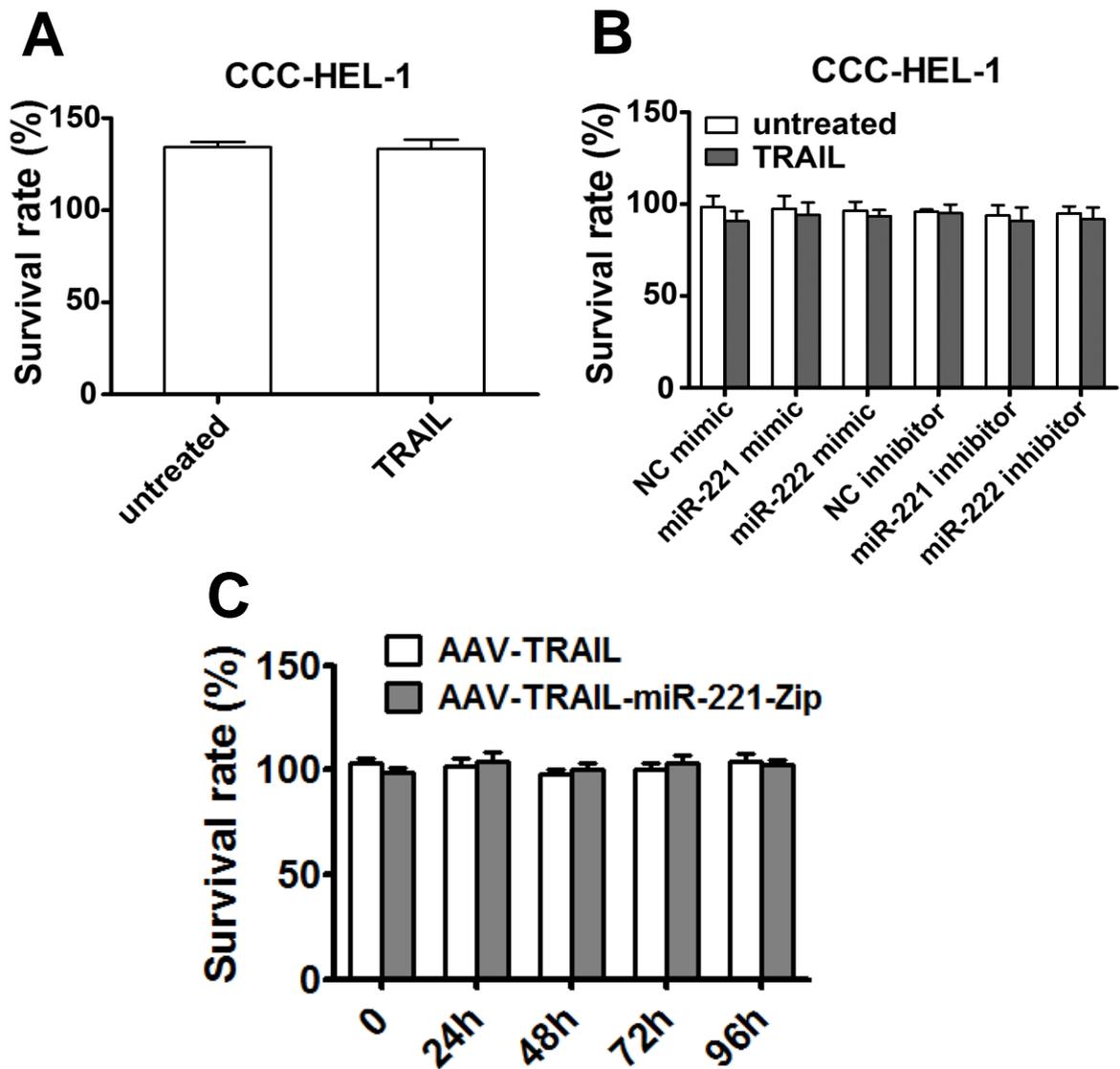


FIGURE S13. Toxicity of strategy of TRAIL combined with miR-221 inhibitors *in vitro*. (A) CCC-HEL-1 cells were treated for 24 hours with 1000ng/ml TRAIL, and cell viability was determined by CCK-8 assay. (B) CCK-8 assay to determine the cell viability treated for 24 hours with 1000ng/ml TRAIL 48 hours after transfected with miR-221/222 mimics or inhibitor. (C) CCC-HEL-1 cells were infected with AAV-TRAIL or AAV-TRAIL-miR-221-Zip for indicated times, and cell viability was determined by CCK-8 assay.

Table S1

| Gene | Primer Sequence |
|-------------|--|
| beta-actin | 5'-3'CATGTACGTTGCTATCCAGGC 5'-3'CTCCTTAATGTCACGCACGAT |
| miR-221 | RT 5'-3' GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGCACTGG ATACGACGAAACCCA 5'-3'GGGGGGGAGCTACATTGTC 5'-3'CAGTGCAGGGTCCGAGGT |
| miR-222 | RT 5'-3' GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGCACTGG ATACGACGACCCAGT 5'-3'GGGGGGGAGCTACATCTGG 5'-3'CAGTGCAGGGTCCGAGGT |
| trail | 5'-3' AGAACGACAAACAAATGGTCCAA 5'-3' GCCAACTAAAAAGGCCCCGA |
| cag | 5'-3'GGCGTGGTGTGCACTGT 5'-3'GTTCCGCCGTGGCAATAG |
| U6 | RT 5'-3'CGCTTCACGAATTTGCGTGTCAT 5'-3'GCTTCGGCAGCACATATACTAAAAT 5'-3'CGCTTCACGAATTTGCGTGTCAT |
| Oligo dT | 5'-3'TTTTTTTTTTTTTTTT |
| miR-125b | RT 5'-3' GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGCACTGG ATACGACTCACAAGT 5'-3'GGGGGGGTCCCTGAGA 5'-3'CAGTGCAGGGTCCGAGGT |
| miR-4288 | RT 5'-3' GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGCACTGG ATACGACGGAAACT 5'-3'GGGGGGGTTGTCTGCTG |

| | |
|----------|--|
| | 5'-3'CAGTGCAGGGTCCGAGGT |
| miR-144 | RT 5'-3' GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGCACTGG ATACGACAGTACATC 5'-3'GGGGGGGGGTACAGTATAG 5'-3'CAGTGCAGGGTCCGAGGT |
| miR-4638 | RT 5'-3' GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGCACTGG ATACGACACTTGTC 5'-3'GGGGGGGACTCGGCT 5'-3'CAGTGCAGGGTCCGAGGT |
| miR-375 | RT 5'-3' GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGCACTGG ATACGACTCACGCGA 5'-3'GGGGGGGGTTTGTTCGT 5'-3'CAGTGCAGGGTCCGAGGT |

Table S2. The IC50 of Huh7, Bel-7402, SMMC-7721 and HepG2 to TRAIL stimulation

| Cell line | IC50 |
|------------------|-------------|
| HepG2 | 5806 ng/ml |
| Bel-7402 | 66 ng/ml |
| SMMC-7721 | 495 ng/ml |
| Huh7 | 5569 ng/ml |

Table S3. The IC50 of eight HCCs in response to TRAIL stimulation.

| HHC cells isolated from tissues | IC50 |
|--|---------------|
| HCC1 | 1663.21 ng/ml |
| HCC2 | 3125.36 ng/ml |
| HCC3 | 303.25 ng/ml |
| HCC4 | 422.37 ng/ml |
| HCC5 | 43.69 ng/ml |
| HCC6 | 4311.48ng/ml |
| HCC7 | 2901.39 ng/ml |
| HCC8 | 497.25 ng/ml |