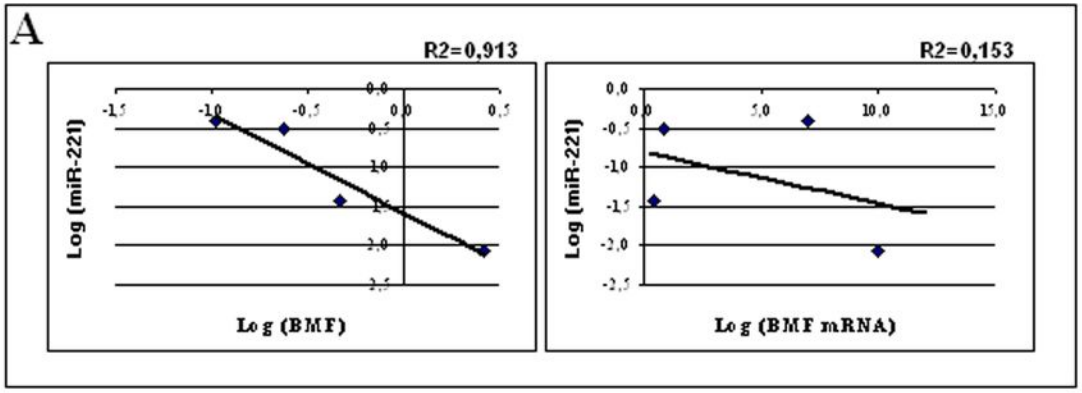
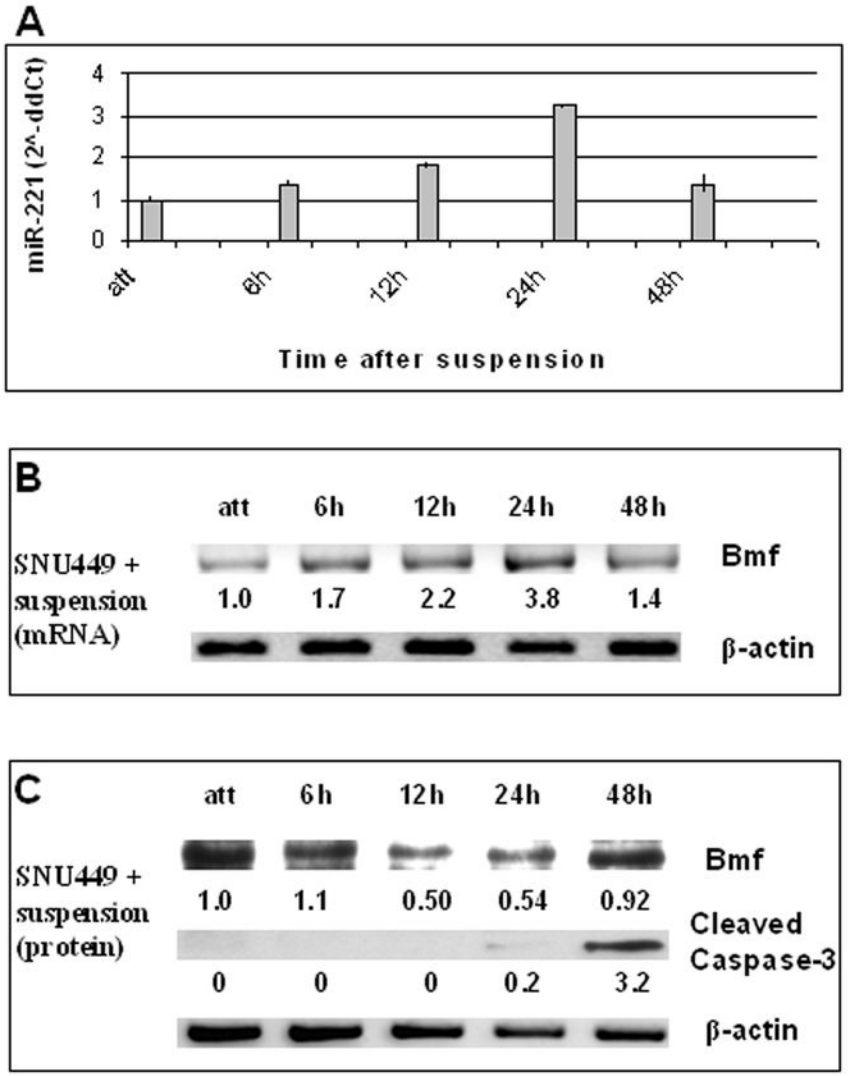
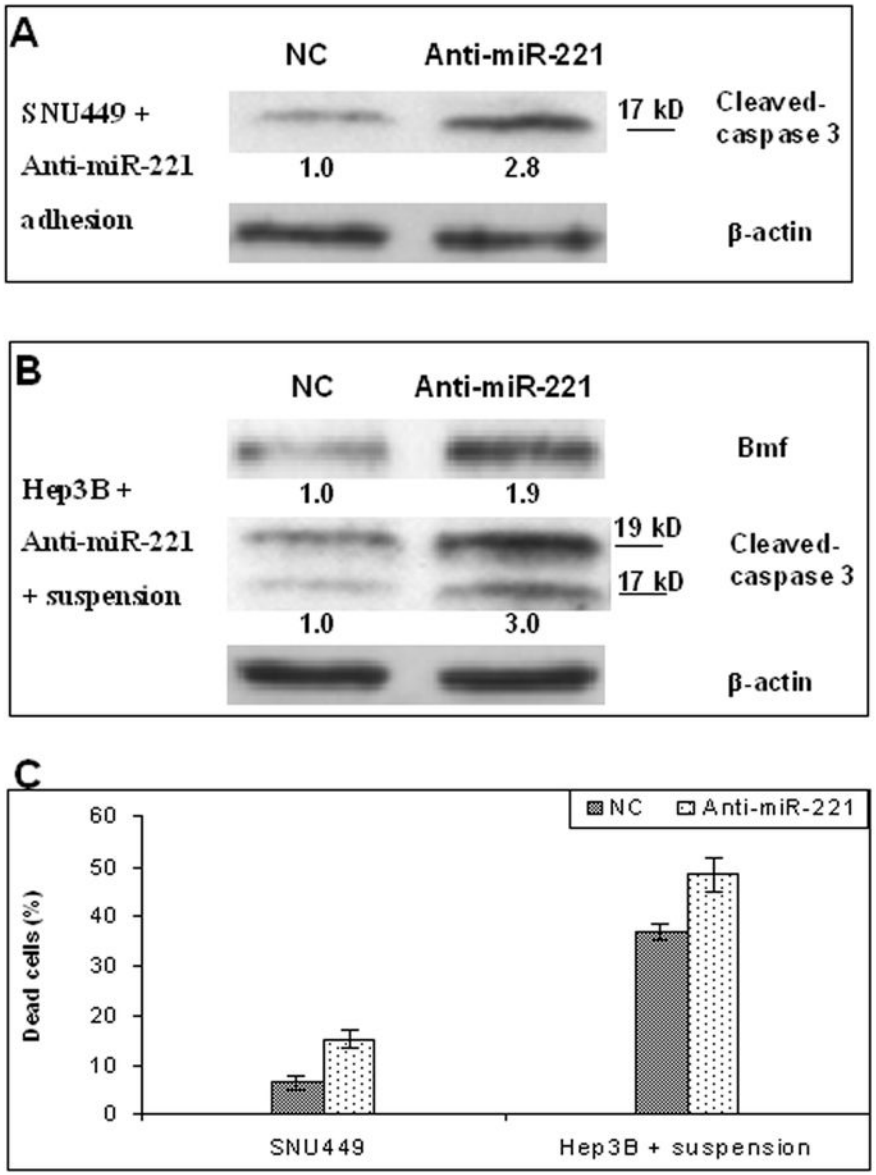
Supplementary_Figure_1



Supplementary_Figure_2



Supplementary_Figure_3



Supplementary Figure Legends

Supplementary Figure S1. Relationship between miR-221 and Bmf protein and mRNA levels.

Inverse correlation between miR-221 and Bmf protein expression in HCC-derived cell lines. Both miR-221 and Bmf values are expressed in a log2 form (left side). No correlation was found between miR-221 and BMF mRNA (right side).

Supplementary Figure S2. MiR-221 and Bmf expression during anoikis of SNU449 cells.

(A) Real-time RT-PCR analysis of miR-221 expression in SNU449 cell line following cell suspension. A progressive increase of miR-221 expression is observed until 24h of non-adherent conditions (3.2-fold icrease), while at 48h following suspension miR-221 expression goes down to approximately basal levels (1.4-fold increase). (B) Semi-quantitative RT-PCR analysis of Bmf mRNA expression in SNU449 cells following cell suspension. Six hours after cell suspension, Bmf mRNA is progressively up-regulated reaching a 3.8-fold increase at 24h. At 48 hours of non-adherent growth, Bmf mRNA levels decrease to 1.4 fold basal levels. (C) Western blot analysis of Bmf and cleaved caspase-3 expression following cell suspension. A progressive decrease of Bmf protein is observed until 24h of non-adherent conditions (2.0-fold decrease), paralleling miR-221 increase. At 48h of suspension, Bmf protein returns to approximately basal levels (0.92-fold) and triggers apoptosis, as shown by the appearance of cleaved caspase-3 related band. At 48 hours, increased levels of Bmf protein are associated with a reduction of miR-221 levels, despite a down-regulation of Bmf mRNA expression. Basal conditions are represented by attached cells (att) in each panel.

Supplementary Figure S3. Anti-miR-221 increases the susceptibility to apoptosis in HCC-derived cell lines grown both in adherent and non-adherent conditions. Anti-miR-221

transfection of SNU449 cell line, in adherent conditions, is associated with an enhanced apoptotic cell death, as shown by a 2.8-fold increase of cleaved caspase 3 expression (A) and with a 2.4-fold increase of dead cells (C), when compared with negative control transfected cells. Anti-miR-221 transfection of Hep3B cells, in non-adherent conditions, is associated with an enhanced apoptotic cell death, as shown by a 1.9-fold and 3.0-fold increase of Bmf and cleaved caspase-3 expression, respectively (B) and with a 1.3-fold increase of dead cell number (C), when compared with negative control transfected cells.