Supplementary Information

Regulation of the expression of the liver cancer susceptibility gene MICA by microRNAs

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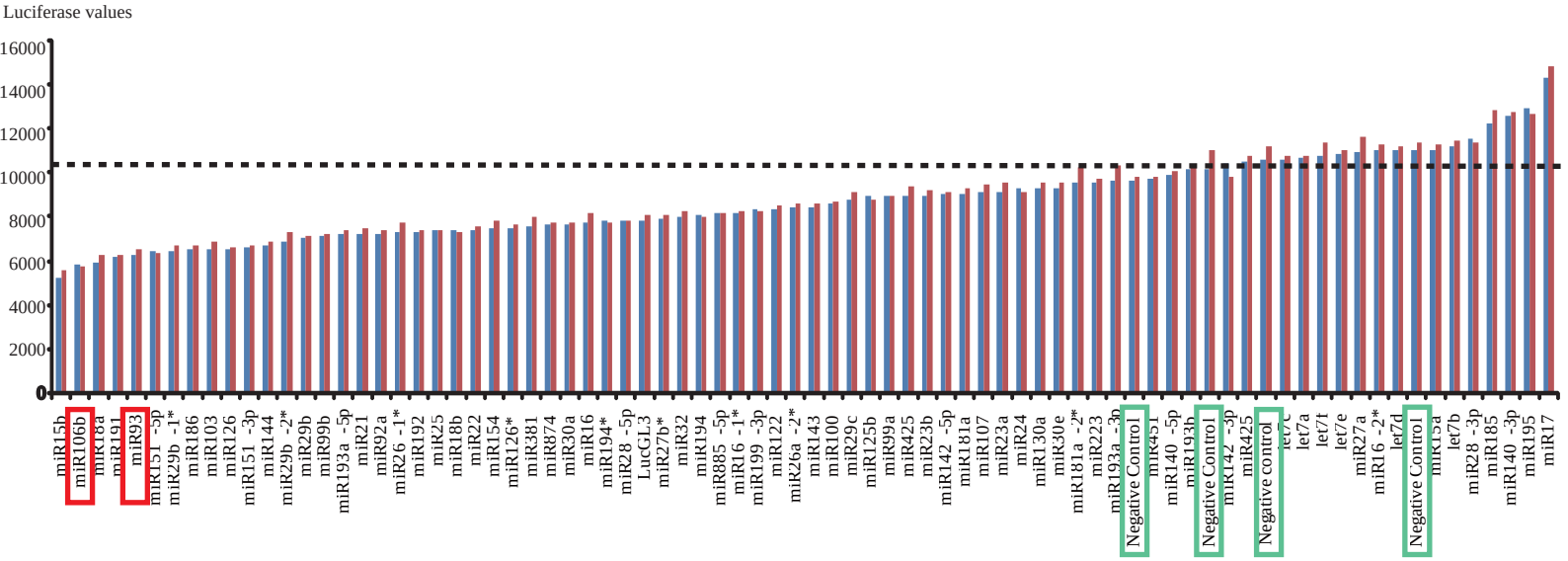
Inventory of Supplementary Information:

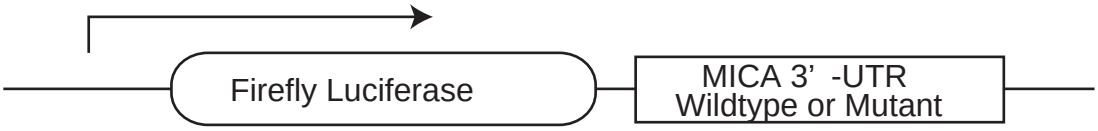
Supplementary Figures (Figures S1-S5)
Supplementary Figure Legends

Supplementary Figure 1



Supplementary Figure 2

a



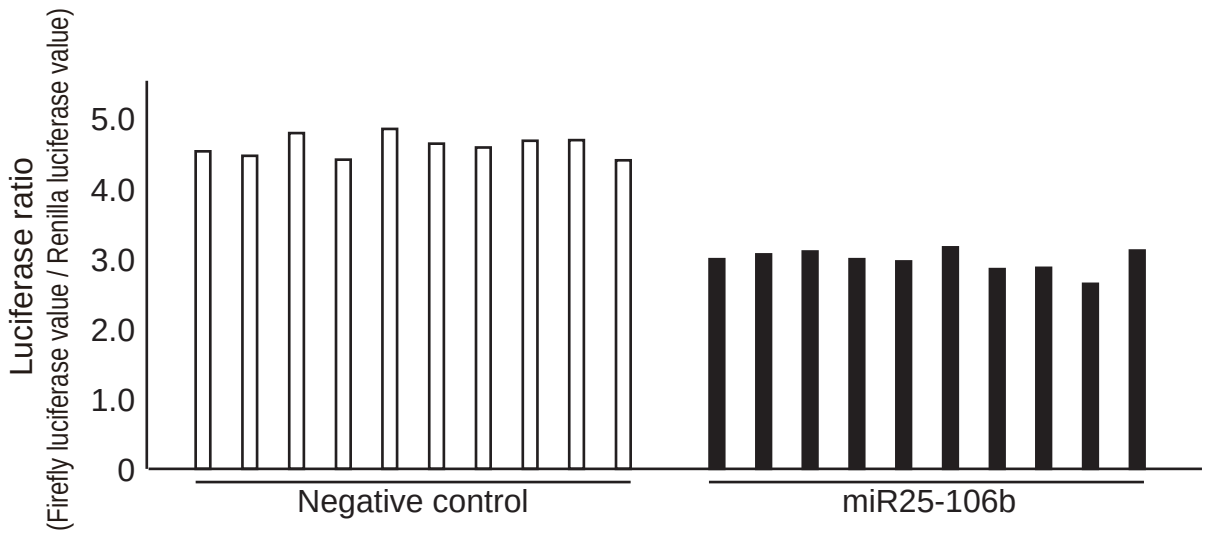
Wildtype 3'UTR (Luc-MICA 3'UTRwt)

5'.....ATTTCAGCCTCTGATGTCAGCTCTTGGGTCCACTGGCTCCACTGAGGGCACCTAGACTCTACAGCC
AGGCGGCTGGAATTGAATTCCCTGCCTGGATCTCACAAGCACTTTCCCTCTTGGTGCCTCAGTTTCCTGA
CCTATGAAACAGAGAAAATAAAAGCACTTATTTATTGTTGTTGGAGGCTGCAAAATGTTAGTAGATATGA
GGCATTTGCAGCTGTGCCATATTAATTGGTGTCATTGTTTTTGTTGTTTTCGTATTATTATTTTTTTTTTTTA
AGACAGAGTCTCAGGCCA 3'

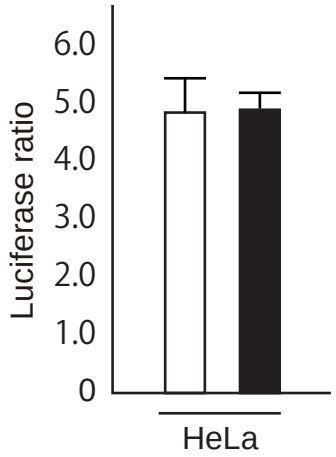
Mutant 3'UTR(Luc-MICA 3'UTRmut)

5'.....ATTTCAGCCTCTGATGTCAGCTCTTGGGTCCACTGGCTCCACTGAGGGCACCTAGACTCTACAGCC
AGGCGGCTGGAATTGAATTCCCTGCCTGGATCTCACAAGGAGTATCCCTCTTGGTGCCTCAGTTTCCTGA
CCTATGAAACAGAGAAAATAAAAGCACTTATTTATTGTTGTTGGAGGCTGCAAAATGTTAGTAGATATGA
GGCATTTGCAGCTGTGCCATATTAATTGGTGTCATTGTTTTTGTTGTTTTCGTATTATTATTTTTTTTTTTA
AGACAGAGTCTCAGGCCA 3'

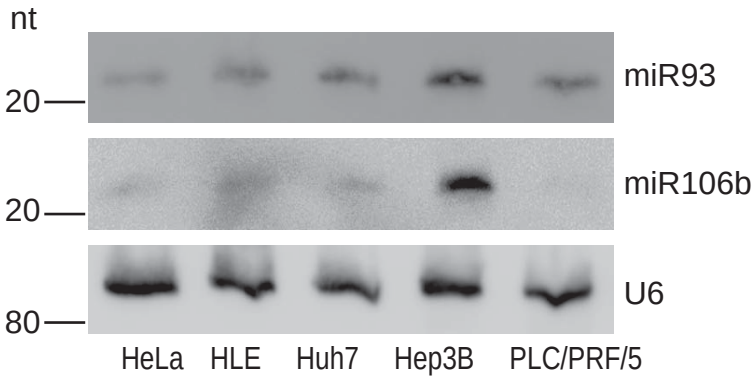
b



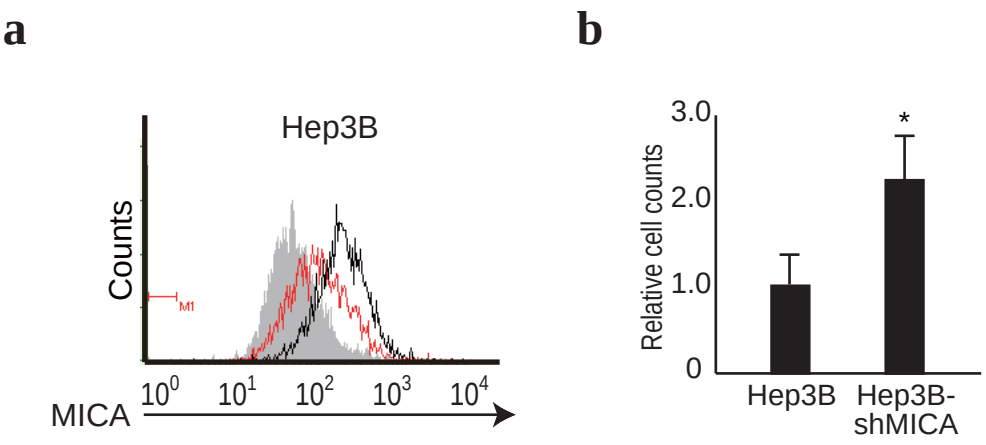
c



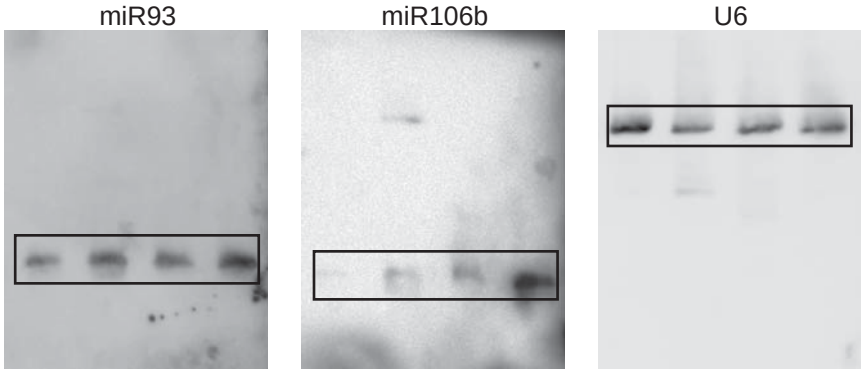
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Screening for miRNAs targeting the MICA 3′UTR.

Seventy-six types of synthetic mature miRNAs were transfected into Huh7 cells that had been transfected with the luciferase-based reporter with the MICA 3′UTR. Twenty-four hours after miRNA library transfection, luciferase values were determined. The experiments were performed in duplicate (blue and red bars). Four artificial oligonucleotides were included as negative controls (green rectangles). The means of negative controls are shown as a dashed line. miR93 and miR106b (red rectangles) were subjected to the subsequent analyses.

Supplementary Figure 2. Luciferase reporter with the MICA 3′UTR.

a, Schematic representation of the luciferase reporter used to investigate the regulatory role of the MICA 3′UTR. The firefly luciferase gene, driven by a CMV promoter, contains the MICA 3′UTR in its 3′-UTR (Luc-MICA 3′UTRwt). For mutants (Luc-MICA 3′UTRmut), three point mutations were inserted in the region complementary to the seed sequences of the possible miRNA target site (underlined black letters). **b,** Representative examples of the variances for the luciferase assays. HeLa cells were transfected with Luc-MICA-3'UTRwt, pGL4-TK, and either an empty control vector (white bar) or miR25-93-106b cluster expression plasmid (black bar). Ten transfections were performed per group. The raw results after dividing firefly luciferase values by renilla luciferase values in each transfection are shown. **c,** The effects of unrelated miRNA expression on MCIA-3'UTR. HeLa cells were co-transfected with Luc-MICA-3'UTRwt, pGL4-TK, and either an empty control vector (white bar) or let-7g expression plasmid as a representative of unrelated miRNAs (black bar). No significant differences were obtained.

Supplementary Figure 3. miR93 and miR106b expression levels.

miR93 and miR106b expression levels were determined by northern blotting. U6 levels were used as a loading control. Representative images from two independent experiments are shown.

Supplementary Figure 4. MICA knocked-down Hep3B cells were more resistant to *in vivo* cell killing. a, Flow cytometry assessment of MICA protein expression in MICA knocked-down Hep3B cells (red line). Isotype IgG was used for background staining (gray shaded) and MICA expression levels in control Hep3B cells are shown (black line). Representative results from two independent experiments are shown. **b,** *In vivo* killing of DiO-labeled Hep3B or MICA-knocked down Hep3B cells, and Dil-labeled HeLa cells (internal control cells), injected together into the tail veins of two mice in each group. Fluorescence intensities were quantified by flow cytometry as the ratio of Hep3B cells to HeLa cells in the lungs. The data from control Hep3B cells were set as 1.0. Data represent the means ± s.d. of two independent experiments. $*p < 0.05$.

Supplementary Figure 5. Full-length blot images for Northern blotting results.

Full-length northern blotting images for Figure 2d.