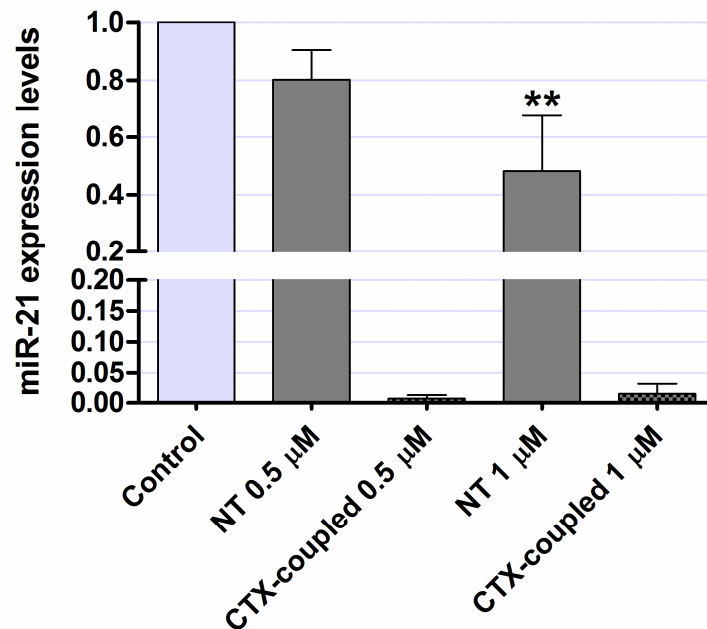


Supplementary Figure 4. Association and internalization of liposomes encapsulating anti-survivin siRNAs in survivin-expressing U87 glioma cells, and effect on survivin mRNA expression. Cells were incubated, for 4 hours at 37°C, with NBD-labeled CTX-coupled (CTX) or nontargeted (NT) liposomes encapsulating anti-survivin siRNAs. Cells were then rinsed twice with PBS and prepared for flow cytometry analysis or stained with DNA-specific Hoechst 33342 (blue) for confocal microscopy analysis. **(a)** Fluorescence intensity plot of cells incubated with 0.5 or 1 μ M of SNALP-formulated anti-survivin siRNAs. Relative fluorescence units (RFU) to control cells (untreated) are indicated for each plot. **(b, c, d, e)** Representative images at 40 \times magnification of U87 cells incubated with **(b, d)** NT or **(c, e)** CTX-coupled liposomes at a final siRNA concentration of **(b, c)** 0.5 and **(d, e)** 1 μ M. Results are representative of two independent experiments. **(f)** Survivin mRNA expression levels in U87 cells, 48 hours after cell incubation with NT or CTX-coupled liposomes encapsulating anti-survivin or scrambled siRNAs, at a final siRNA concentration of 0.25 or 0.5 μ M (n=2). Cells transfected with lipoplexes (prepared with siRNAs and Lipofectamine as described in Materials and Methods) were used as positive control. Survivin expression levels, normalized to the reference HPRT1, are presented as relative

expression values to control untreated cells. Values are presented as means \pm standard deviation (n=3). *** p<0.001 compared to cells incubated with a similar amount of NT SNALP-formulated siRNAs. # p<0.05 compared to cells incubated with a similar amount of scrambled siRNAs. + p<0.05 compared to cells incubated with a similar amount of NT SNALP-formulated siRNAs. Scale corresponds to 20 μ m.



Supplementary Figure 5. MiR-21 expression in U87 GBM cells following incubation with CTX-coupled or nontargeted liposomes encapsulating anti-miR-21 oligonucleotides. MiR-21 expression levels in U87 cells, 48 hours after cell incubation with CTX-coupled or nontargeted (NT) liposomes encapsulating anti-miR-21 oligonucleotides (n=3). MiR-21 expression levels, normalized to the reference snord44, are presented as relative expression values to control untreated cells. Values are presented as means \pm standard deviation (n=3). ** p<0.01 to cells that were not exposed to liposomes.