# Supporting Information

## Garbuzenko et al. 10.1073/pnas.1004604107

### SI Materials and Methods

**Cell Line.** A549 human lung adenocarcinoma epithelial cell line transfected with luciferase was purchased from Xenogen. Cells were cultured in RPMI medium 1640 (Sigma) supplemented with 10% FBS (Fisher Chemicals) and 1.2 mL/100 mL penicillin–streptomycin (Sigma). Cell were grown at 37 °C in a humidified atmosphere of 5% $CO_2$ (vol/vol) in air. All experiments were performed on cells in the exponential growth phase.

**Cytotoxicity.** The cellular cytotoxicity of all studied formulations was assessed using a modified MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. To measure cytotoxicity, cells were separately incubated in a 96-well microtiter plate with different concentrations of all studied formulations with appropriate controls, which resulted in a total of 20 separate series of experiments.

**Expression of Targeted Genes and Proteins.** The expression of *MRP1, BCL2, CASP9,* and *CASP3* genes was measured using quantitative RT-PCR. Gene expression was calculated as a percent of internal standard ($\beta_2$-microglobulin). To identify the presence of MRP1 and BCL2 proteins, immunohistochemical staining was conducted on paraffin-embedded slides of tumor tissue. Slides were deparaffinized in xylene for 5 min followed by progressive rehydration in 100%, 95%, 70%, and 50% ethanol for 3 min during each step. Endogenous peroxidase activity was blocked by incubating slides in 3% $H_2O_2$ solution in methanol at room temperature for 10 min and washing in 300 mL PBS two times for 5 min. The slides were then stained with antimouse monoclonal antibodies for MRP1 (rat IgG2a) conjugated with Alexa-Fluor®647 (BioLegend, catalog no. 124810) and for BCL2 (rat IgG2a) conjugated with FITC (BioLegend, catalog no. 633502) by incubating for 1 h in PBS buffer containing antibodies in concentration of 1 μL antibodies per 200 μL PBS, washed in 300 mL PBS two times for 5 min, and visualized by fluorescence microscopy.

**Apoptosis.** Apoptosis induction in lungs with tumor and other organs was measured using Cell Death Plus ELISA and TUNEL kits (Hoffmann-La Roche).

**Histopathologic Analysis.** After the animals were euthanized, the tumors and organs were extracted and immediately fixed in 10% phosphate-buffered formalin. Samples were subsequently dehydrated and embedded in Paraplast. Sections (5 μm) were cut, stained with H&E, and analyzed.

**Quantitative Analysis of Tumor Size, Mass, and Volume.** To quantitatively estimate tumor size, mass, and volume in the lungs of live animals, a special series of experiments was carried out. In these experiments, A549 lung cancer cells transfected with luciferase were inoculated into the flanks of nude mice, tumor growth was monitored by the IVIS imaging system (Fig. S1*A*), and tumor size was measured by a caliper. A strong positive correlation was found between the two sets of data (Fig. S1*B*). The tumors were excised and weighed, and their weights were compared with their size and bioluminescence measured in live animals before they were killed (Fig. S1*C*). Similarly, a strong linear correlation was found between the actual tumor mass and volume on the one hand and the intensity of bioluminescence on the other hand (Fig. S1*C*). Taken together, these data confirm that the measurement of bioluminescence of tumor cells can be used for quantitative monitoring the growth of the tumor. The established correlation equation was further used to estimate the total volume of lung tumor in live mice. In addition, these data were compared with those obtained using an ultrasound imaging system (Fig. 3*B*). A strong positive correlation was found between the data obtained using these two distinctly different techniques. In addition to tumor progression, we used the IVIS system to evaluate the organ distribution of DDS after i.v. and inhalation instillations using liposomes labeled with the near-infrared cyanine dye Cy5.5. Experimental in vitro data showed that this approach allowed reliable detection of the fluorescent dye in a wide range of its concentrations in the solution (1–1,000 nM) (Fig. S1*D*).

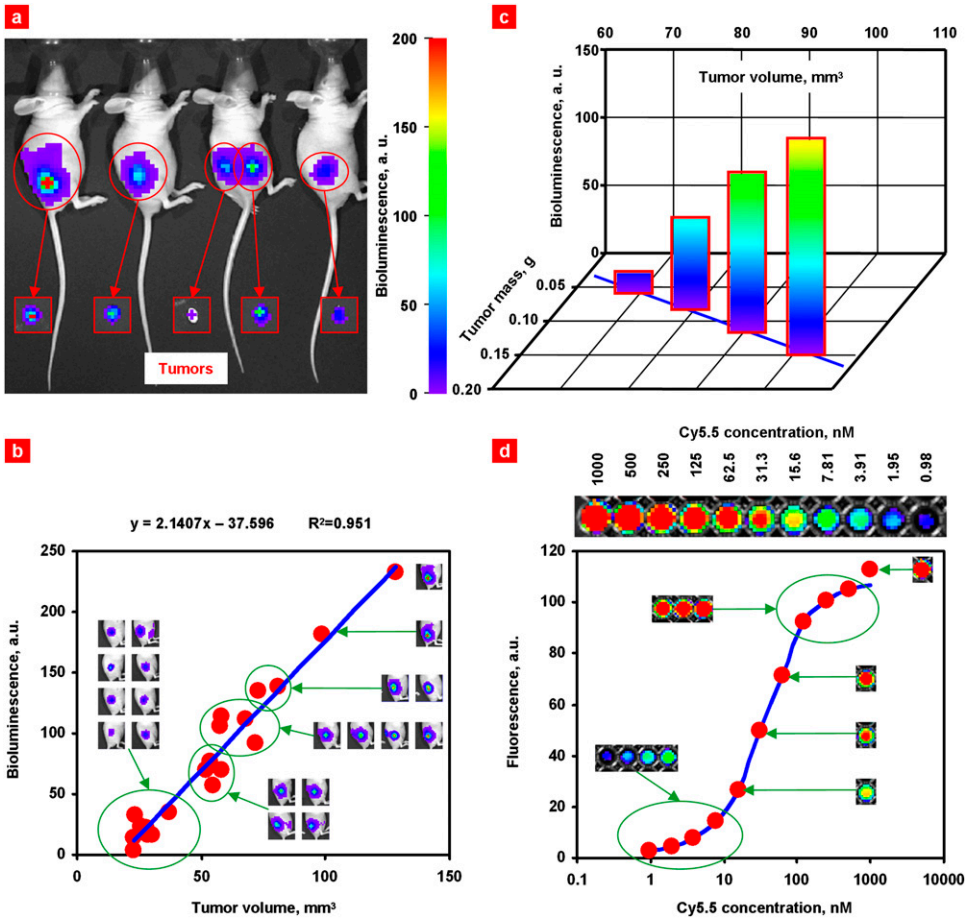


**Fig. S1.** Visualization by IVIS imaging system of lung cancer cells transfected with luciferase and liposomes labeled with fluorescent dye. (*A*) Typical image of a nude mice bearing s.c. xenograft of human A549 lung cancer cells transfected with luciferase. Intensity of fluorescence is expressed by different colors, with blue reflecting the lowest intensity and red indicating the highest intensity. (*B*) Correlation between tumor volume measured by a caliper and the intensity of bioluminescence. (*C*) Correlation between volume/mass of tumors measured by a caliper and weighed after excision and the intensity of bioluminescence. (*D*) Typical image of a microtiter plate row loaded with different dilutions of Cy5.5, and correlation between the Cy5.5 dye concentration and fluorescence intensity.

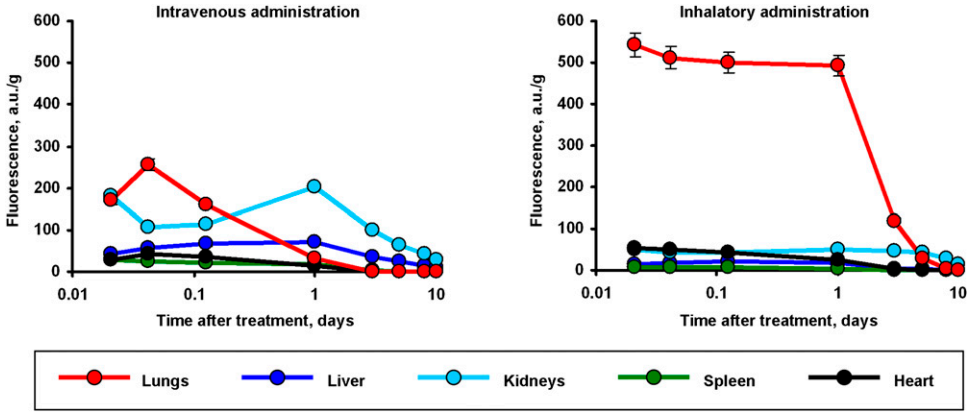


**Fig. S2.** Inhalation delivery enhances lung exposure to liposomal antisense oligonucleotides (ASO) and limits their content in other organs. ASO were labeled with FITC and delivered by i.v. route or inhalation into mice. Fluorescence of ASO in different organs was measured using IVIS Xenogen imaging system and expressed per gram organ weight. Mean ± SD are shown.