Supplementary Data for NAR-00971-C-2009:

Anti-tumor activity of splice-switching oligonucleotides

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SUPPLEMENTARY METHODS

Detection of Bcl-x_S levels. B16F10 cells were plated at 2.0×10^5 cells/plate in 60 mm^2 plates. Cells were transfected with 4 ug Bcl-x_S plasmid DNA (pcDNA3.1-Bcl-x_S graciously provided by Dr. Reuven Stein, Tel Aviv University, Tel Aviv, Israel) using LipoFectamine 2000 according to the manufacturer's protocol. Approximately 24 hours later, the transfected cells were rinsed twice with PBS and suspended using Trypsin-EDTA. B16F10 cells tranfected with pcDNA3.1-Bcl-x_S were then diluted in un-transfected B16F10 cells at various ratios: 1:2, 1:5, 1:10, 1:25, 1:50 and 1:100. Cells were then subjected to RNA and protein isolation and analysis as described in the Methods section of the paper.

Preliminary Animal Studies. Preliminary animal experiments were carried out as described in the Methods section of the paper, except with a different dosing schedule. Seven days after tumor inoculation, tumor-bearing mice were injected i.v. with NP formulated with Bcl-x SSO at 2.4 mg/kg either (a) everyday for a total of 9 injections, or (b) every other day for a total of 5 injections. The negative control group received no treatment. Animals were euthanized one day following the final injection (day 16 after tumor inoculation). RNA was isolated from tumor nodules and analyzed by RT-PCR as described in the Methods section of the paper.

SUPPLEMENTARY FIGURES:

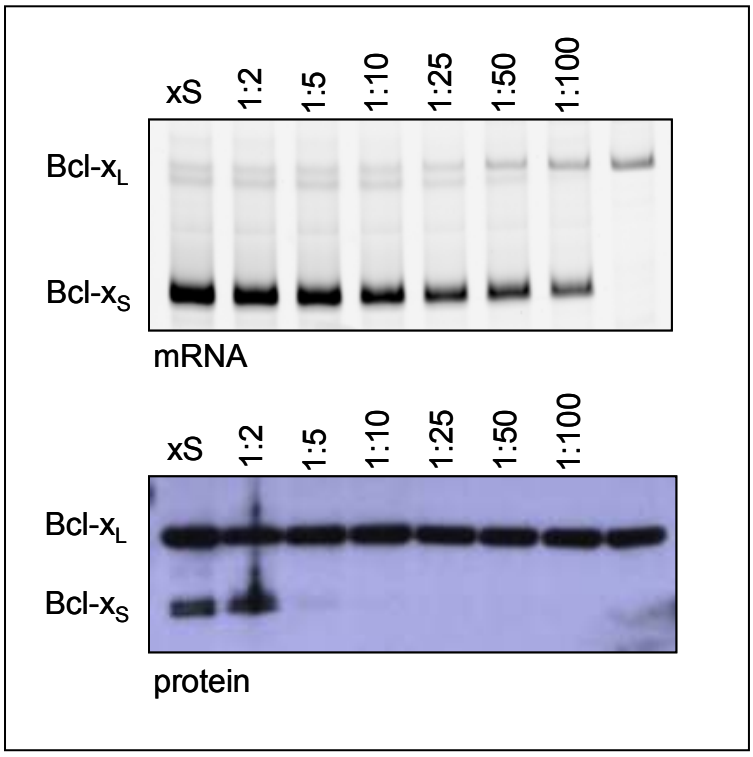

Figure S1. Bcl-x_S detection by RT-PCR and Western Blot. B16F10 cells transfected with pcDNA3.1-Bcl-x_S were diluted with untransfected cells at various ratios (undiluted, 1:2, 1:5, 1:10, 1:25, 1:50, 1:100). RNA and protein were isolated and analyzed by RT-PCR and Western Blot, respectively. Bcl-x_S mRNA was readily detected even at the 1:100 dilution, though it was not detected in untransfected cells. Bcl-x_S protein could be detected up to the 1:5 dilution, but was not seen in more dilute samples. The level of Bcl-x_S mRNA corresponding to the 1:5 dilution is greater than can achieved endogenously through alternative splicing.

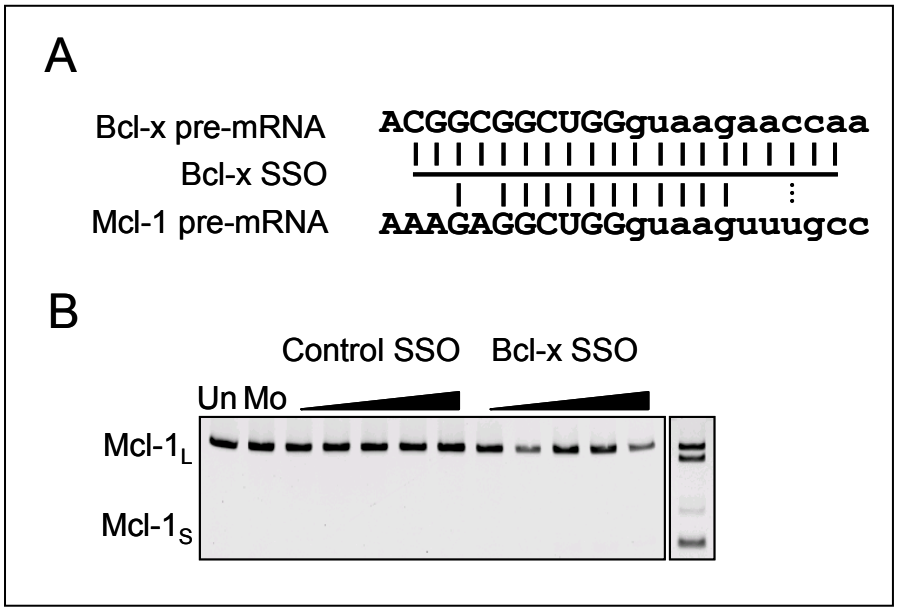

Figure S2. Bcl-x SSO does not affect Mcl-1 alternative pre-mRNA splicing. (A) The Bcl-x and Mcl-1 share a high degree of sequence conservation at the 5′ splice site of exon 2. As a result the Bcl-x SSO matches 13 out of 20 bases in Mcl-1 pre-mRNA (including one G-U pair). (B) RNA from Bcl-x SSO-transfected B16F10 cells was subjected to RT-PCR using primers flanking exon 2 of mouse Mcl-1. Mcl-1 pre-mRNA can be alternatively spliced to produce a transcript that lacks exon 2. However, the Bcl-x SSO fails to redirect Mcl-1 splicing, despite matching 13 out of 20 bases in Mcl-1. SSO concentrations used were 4, 11, 33, 100 and 300nM.

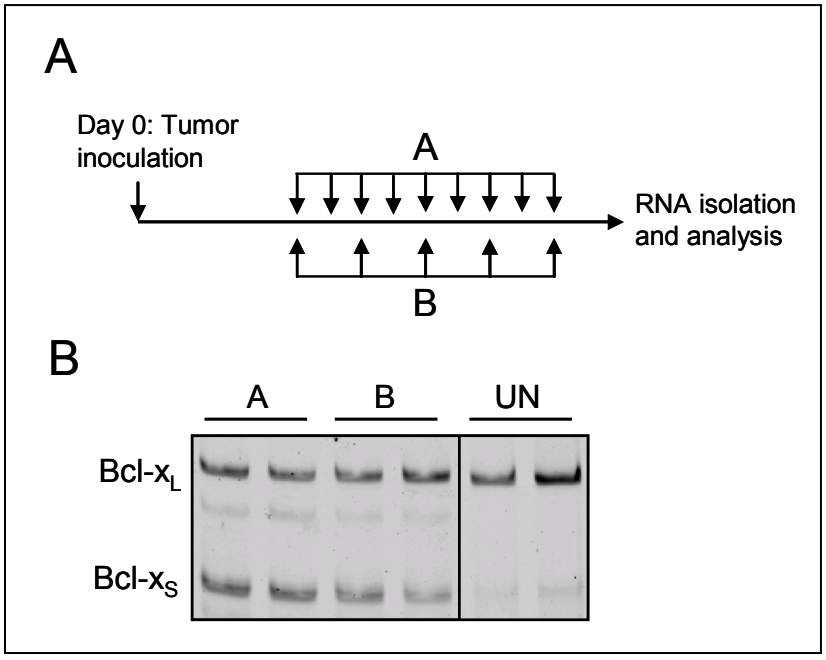

Figure S3. Increasing the number of doses of the Bcl-x SSO NP formulation results in increased Bcl-x splice-switching. (A) Bcl-x SSO NP was systemically delivered (tail-vein injection) every day (9 injections, group A) or every over day (5 injections, group B) to mice bearing B16F10 tumors. (B) RT-PCR analysis of Bcl-x pre-mRNA isolated from lung tumor nodules from mice injected with 2.4 mg/kg NP formulated with Bcl-x SSO every day (9 injections, group A) or every over day (5 injections, group B) revealed robust Bcl-x splice-switching.

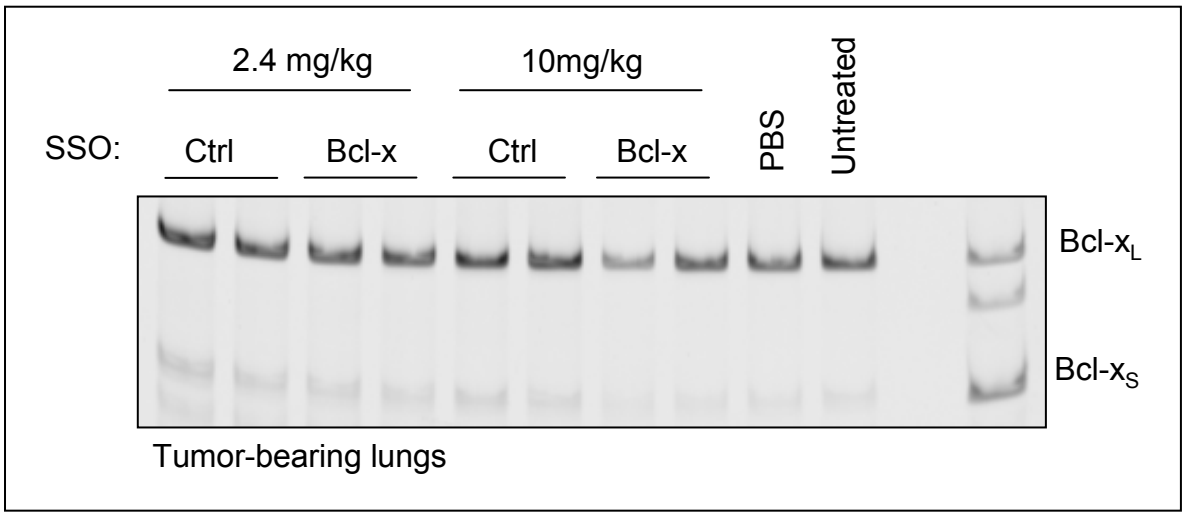

Figure S4. Systemically-delivered free Bcl-x SSO has no effect on Bcl-x pre-mRNA splicing in tumor xenografts. Tumor-bearing animals were injected with vehicle only (PBS) or with free SSO (control SSO or Bcl-x SSO) in PBS at 2.4 or 10 mg/kg on days 3-6 following tumor inoculation. On day 7 animals were euthanized and RNA from tumor-bearing lungs was isolated and analyzed by RT-PCR.