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Supplemental Information

6BIO Enhances Oligonucleotide Activity in Cells: A Potential Combinatorial Anti-androgen Receptor Therapy in Prostate Cancer Cells

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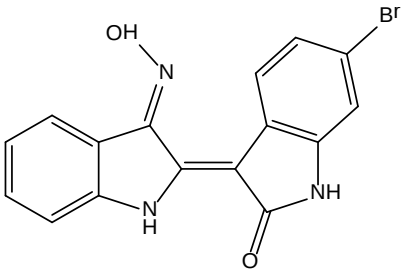
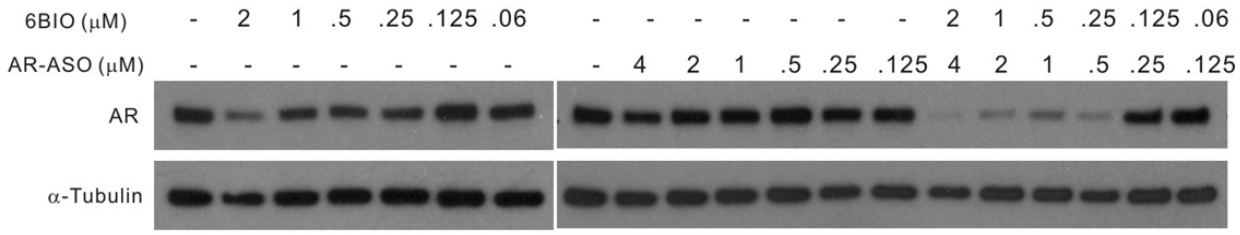
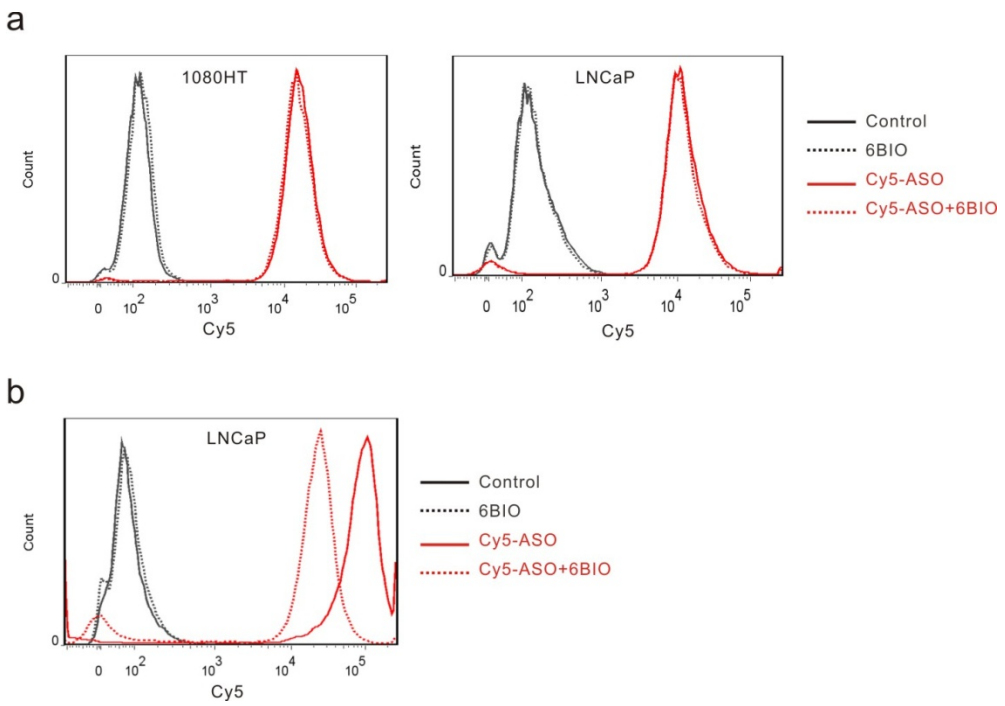


Supplementary Figure S1. The molecular structure of 6BIO. 6-Bromo-indirubin-3'-oxime; its molecular formula is C16H10BrN3O2.

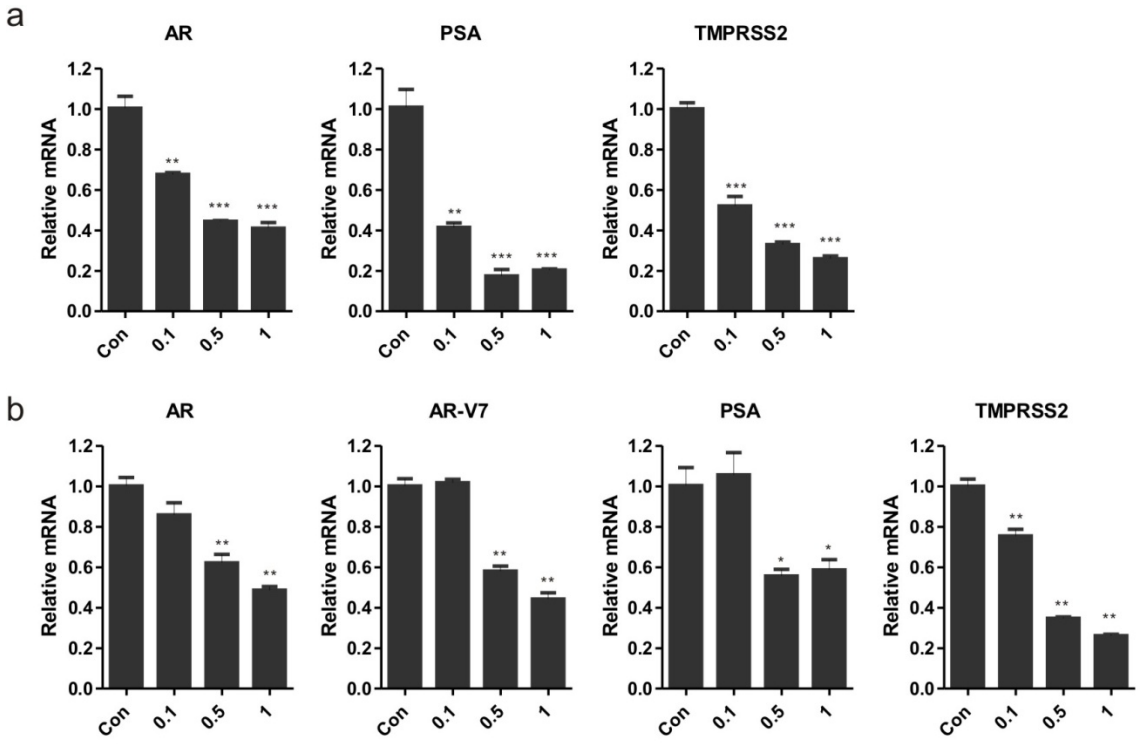


Supplementary Figure S2. Fixed concentration ratios of AR-ASO and 6BIO demonstrate synergistic AR suppression. Representative Western analysis of lysates harvested from LNCaP cells treated with increasing or decreasing concentrations of 6BIO and AR-ASO, as indicated. Three biological replicates demonstrated analogous results. Values of protein reduction for all Westerns were calculated with Image J and normalized to the α-Tubulin as the loading control and to the control, untreated cells. The values were used for the determination of the CI by the Chou-Talalay method [35]; the resulting graph is shown in Figure 5e.

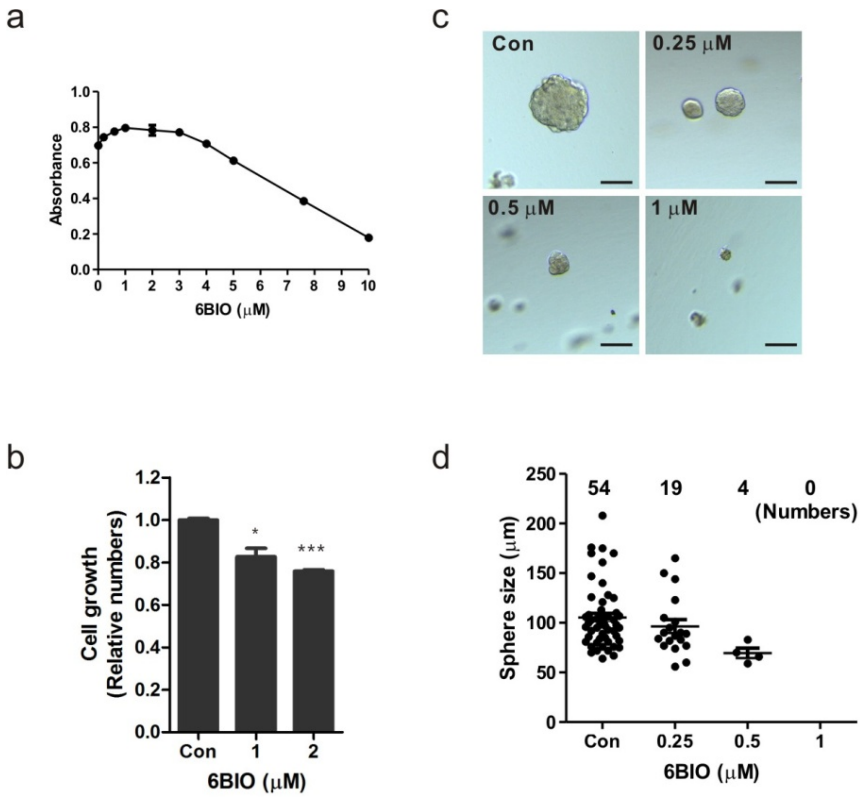


Supplementary Figure S3 6BIO does not enhance the cellular uptake of Cy5-labeled PS LNA gap-mers.

(a) A Cy5-labeled PS LNA gap-mer (Cy5-ASO) with or without 1 μM 6BIO was delivered at 200 nM final concentration by gymnosis to HT1080 or LNCaP cells for 3 hr prior to flow cytometry. Non-treated cells were used as the control. **(b)** The identical experiment as in **a** was extended for 12 hrs in LNCaP cells prior to flow cytometry. Prolonged treatment (12 hrs) with 1 μM 6BIO reduced the uptake of Cy5-ASO in LNCaP cells relative to the non-treated control.



Supplementary Figure S4. 6BIO represses AR mRNA expression and inhibits AR signaling in prostate cancer cells in a dose-dependent manner. Determination of AR, PSA, TMPRSS2 and AR-V7 relative expression by RT-PCR of total RNA derived from **(a)** LNCaP cells and **(b)** 22Rv1 cells, treated with increasing concentrations of 6BIO for 24 hours. Values were normalized to β-actin mRNA expression and expressed as the mean ± s.d., n = 3. Student's *t*-test: *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$. The experiments were repeated two times with similar results.



Supplementary Figure S5. 6BIO inhibits LNCaP cell growth. (a) MTS assay of LNCaP cells treated with increasing concentrations of 6BIO. Data were expressed as the mean ± s.d., n = 3. **(b)** Relative number of LNCaP cells established by cell counting with a hemocytometer. 700,000 LNCaP cells were cultured in 60-mm dishes and treated with 1 or 2 μM 6BIO for three days. Untreated cells served as the control for this experiment. Values were normalized to the control number and expressed as the mean ± s.d., n = 3. Student's *t*-test: *, $p < 0.05$; ***, $p < 0.001$. **(c)** Representative growth of LNCaP microspheres in Matrigel after treatment for 12 days with the indicated increasing concentrations of 6BIO. **(d)** Tumor microsphere numbers (x-axis) and sizes (y-axis) following the 6BIO treatment as indicated in **c**. A tumor microsphere was defined as a colony with a diameter greater than 50 μM. Scale bar, 100 μM.