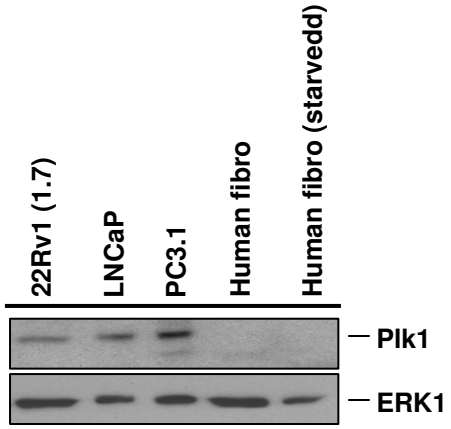
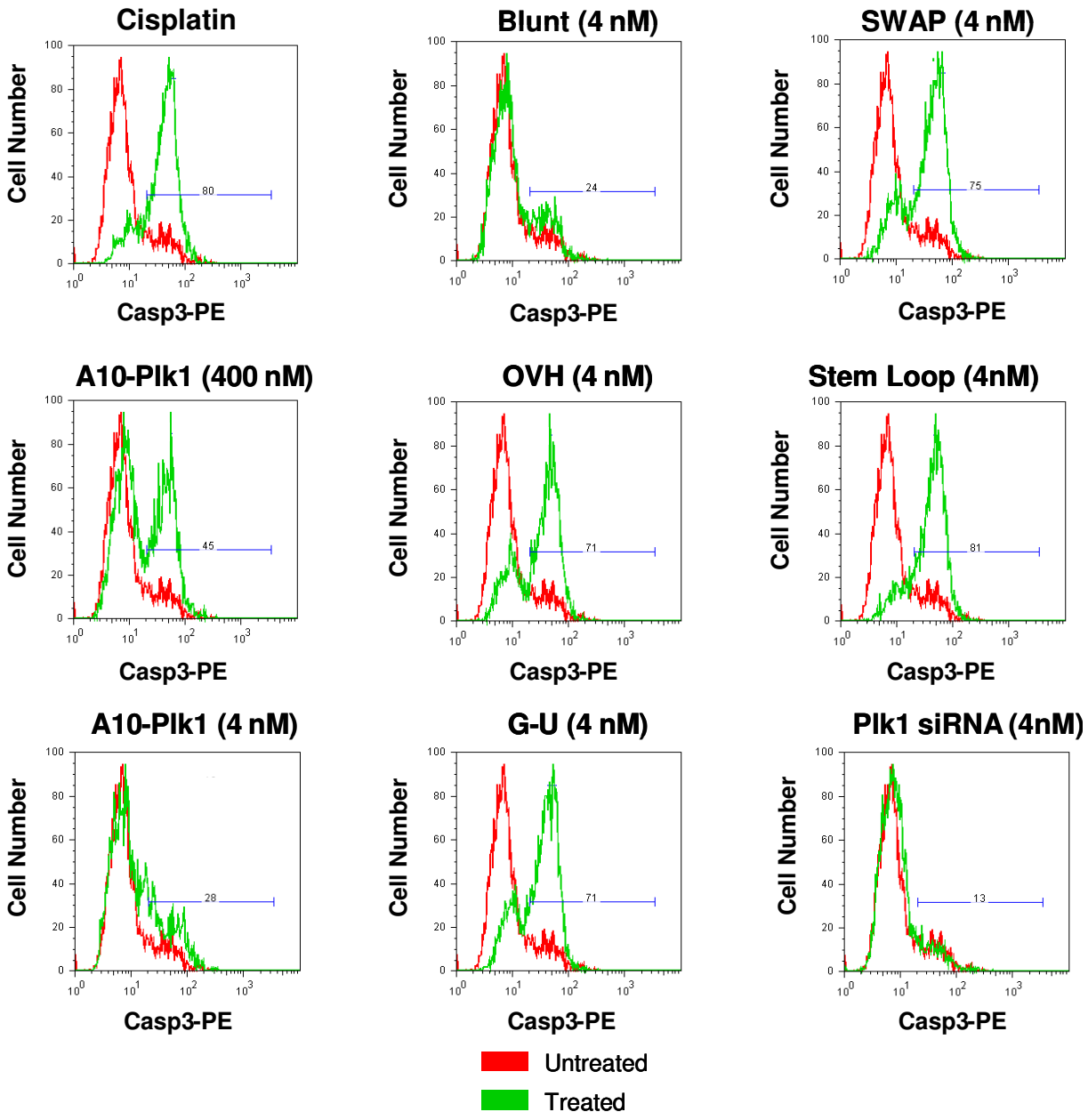
**Supplementary Figure 1**



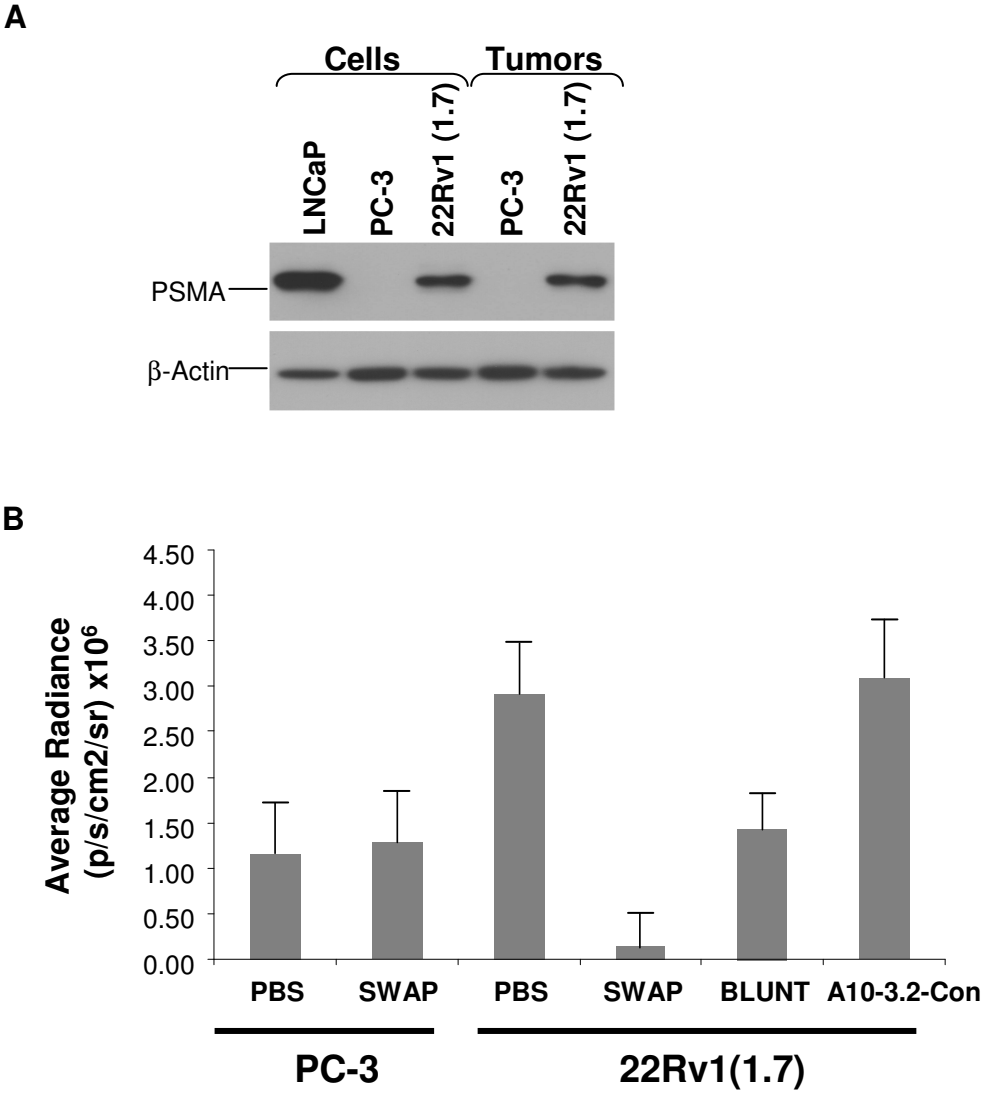

**Supplementary Figure Legend 1. Expression of Plk1 in prostate cancer cells.** Three prostate cancer cell lines 22Rv1(1.7), LNCaP, and PC-3 cells were grown to confluence in a 60mm dish and lysed with RIPA buffer containing protease inhibitors. Lysates were resolved on a PAGE gel, transferred to a PVDF membrane, and blotted for human Plk1 with a specific antibody. Normal human fibroblasts (fibro) were used as a control for this assay. Human fibroblasts were either starved for 48h with media containing 0.2% serum or grown asynchronously. In normal cells Plk1 expression peaks during mitosis and is at its lowest during quiescence whereas cancer cells have intrinsically high levels of Plk1 expression. ERK1 was used as a loading control.

## Supplementary Figure 2



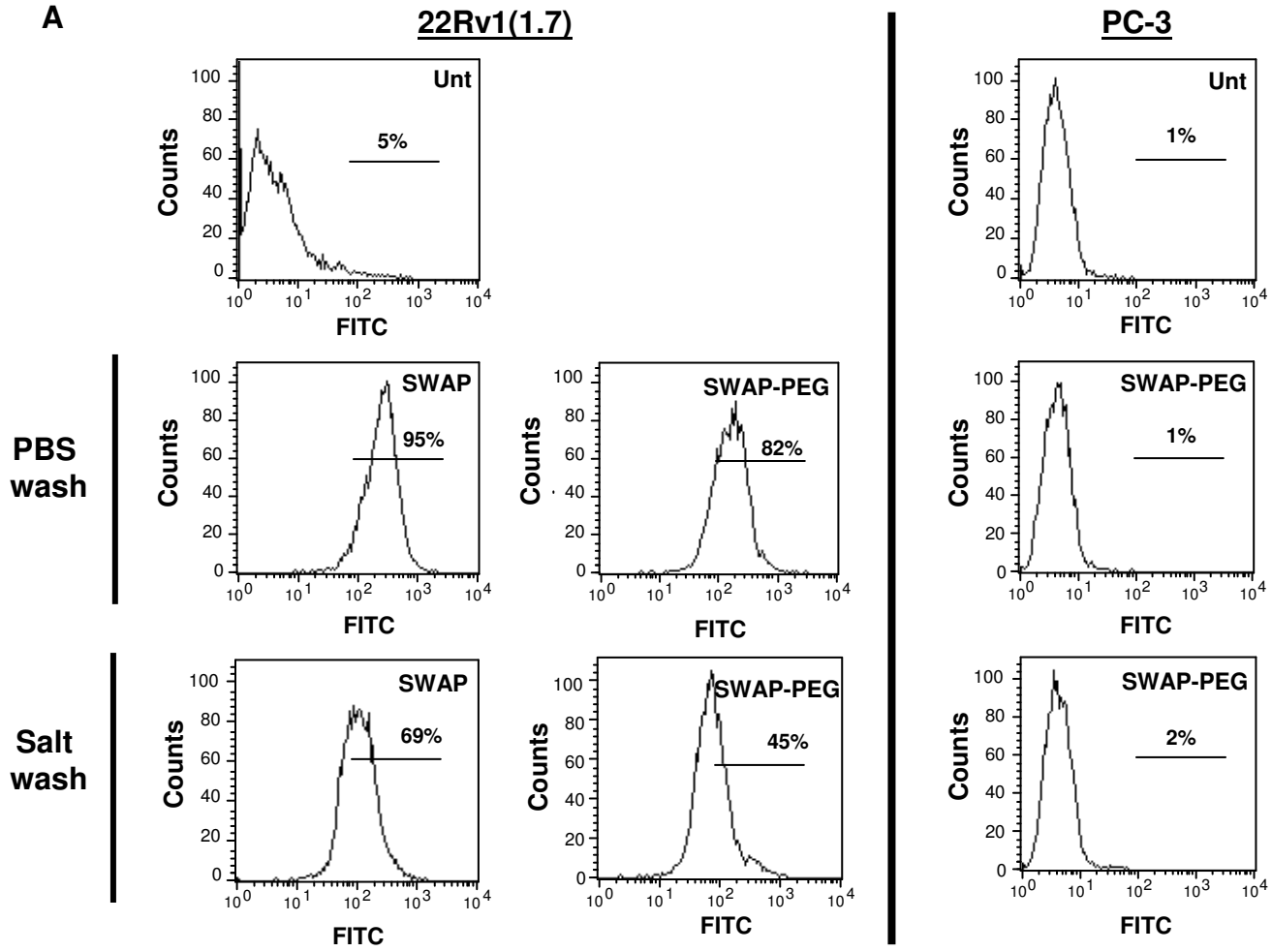

**Supplementary Figure Legend 2. Effect of Optimized PSMA chimeras on prostate cancer cell viability**. 22Rv1(1.7) PSMA-positive prostate cancer cells were treated over the course of 5 days (Day 1 and Day 3) with either 400 or 4nM of each chimera. Cells were collected on Day 5, stained with an antibody against active caspase 3, and processed for flow cytometry. Cisplatin was used as a positive control for this assay. UNT, untransfected; Plk1 siRNA duplex, A10-Plk1, first generation chimera; BLUNT, truncated version of first generation chimera; OVH, truncated version of first generation chimera containing 2nt overhangs at the 3' end; G-U, same as OVH chimera but with addition of a G-U wobble at the 5' end of antisense strand; SWAP, same as OVH but with sense and anti-sense strands swapped; Stem Loop, a hairpin RNA composed of the PSMA aptamer (loop) and a Plk1 siRNA (stem) (see Figure 1).

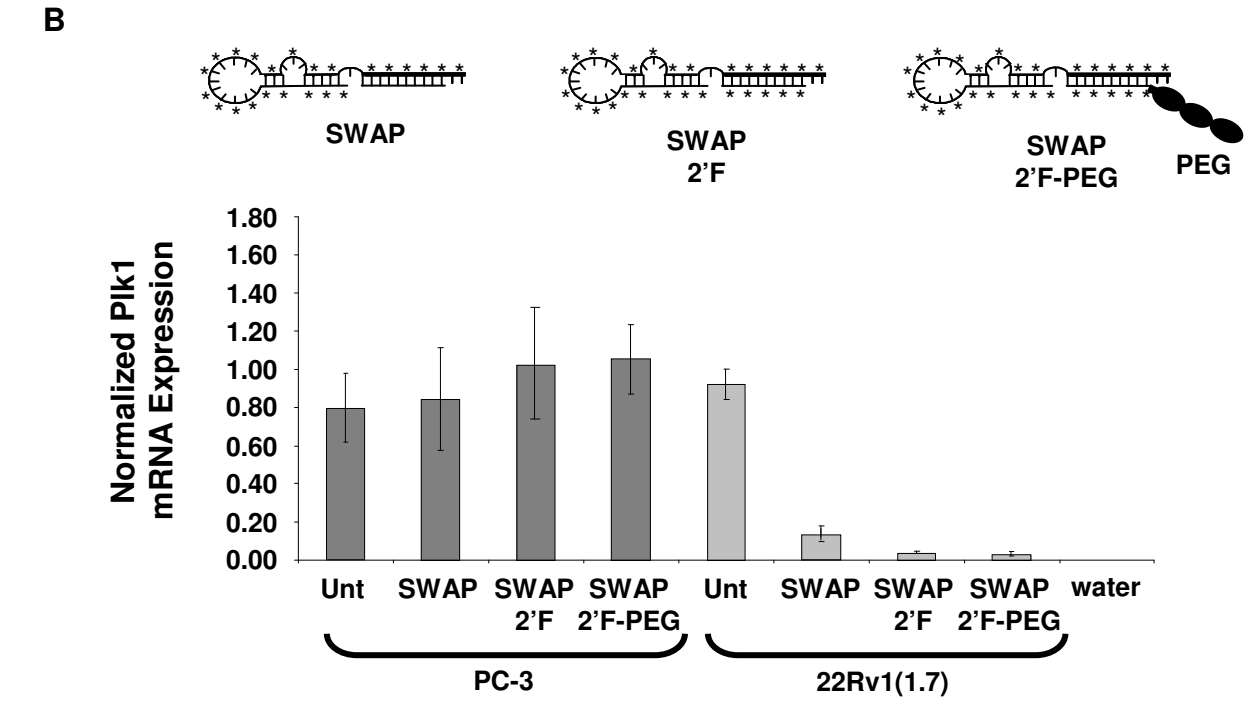
**Supplementary Figure 3**




**Supplementary Figure Legend 3. Assessment of PSMA expression and tumor volume measurements following treatment with PSMA-Plk1 chimeras using BLI. (A)** Protein lysates of prostate cancer cell lines 22Rv1(1.7), LNCaP, and PC-3 and prostate tumors derived from these cells were resolved on an SDS-PAGE gel, transferred to a PVDF membrane, and probed for human PSMA with a specific antibody and β-Actin as a loading control. **(B) Tumor volume measurements using Living Image Software v2.50 (Xenogen).** PSMA-positive (22Rv1) or PSMA-negative (PC-3) prostate cancer cells were injected into the flanks of nude (nu/nu) mice two weeks prior to treatment with optimized chimeras. After tumors reached ~~0.4 $cm^3$ in volume animals were injected (i.p) daily for 10 days with saline (PBS) or 1nmol of the RNA chimeras (BLUNT, SWAP, or A10-3.2-Con). On Day 10 of treatment BLI images of the treated animals were obtained. Tumor volume was determined by placing a circular region of interest (ROI) around each tumor site and total flux quantified using Living Image Software v2.50 (Xenogen) with units photons/sec/$cm^2$/sr. Average tumor volumes were plotted for each treatment group. Animals were sacrificed 2 days after the last treatment. n ≥ 10 mice per treatment group.

**Supplementary Figure 4**



**Supplementary Figure 4. Targeted specificity and efficacy of PEGylated SWAP chimera (SWAP-PEG). A)** PEG-modified SWAP chimera (SWAP-PEG) selectively internalizes into PSMA-positive cells. 22Rv1(1.7) PSMA-positive and PC-3 PSMA-negative prostate cancer cells were incubated at 37$^{o}$C with fluorescently labeled RNA chimeras. Cells were washed with PBS alone or PBS plus 0.5M NaCl (Salt wash) to remove surface bound RNAs. The amount of fluorescently labeled RNAs that bound and/or internalized into cells was quantitated using flow cytometry. **B)** PEG-modified SWAP chimera effectively silences PLK1 mRNA expression following internalization into PSMA-positive prostate cancer cells. 22Rv1(1.7) and PC-3 cells were incubated with media containing 100nM of either the SWAP, SWAP-2'F or SWAP-2'F-PEG chimeras for 48h. Cells were then processed for total RNA and quantitative RT-PCR was performed to assess Plk1 mRNA levels in treated cells. Plk1 mRNA levels were normalized against GAPDH.