Supplemental figure legends

Supplemental Figure 1. The effects of G1T28 are due to CDK4/6 inhibition. A. VCaP and 22Rv1 cells were plated in media containing 10% FBS and increasing concentrations of G1T28 (10^{-10}-10^{-6} M) for 24 hours. Following treatment cells were fixed, permeabilized and stained for AR, and Actin. Cells were imaged using the LI-COR Clx and densitometry calculated from the LI-COR Image Studio software. **B.** VCaP cells were plated in phenol red free media containing 10% charcoal stripped serum for 48 hours before treating with 1nM R1881 in the absence or presence of 1μM G1T28 for 24 hours. After treatment, RNA was isolated, reverse transcribed and analyzed for the expression of the AR target genes ORM and PSA. Data was calculated using the $\Delta\Delta C_t$ method. **C.** VCaP and 22Rv1 cells were plated in media containing 10% FBS and treated with increasing concentrations of G1T28 (10^{-8}-10^{-6} M) for 24 hours. Following treatment, protein was isolated and immunoblotted for phospho-Rb (S807/811), total Rb, cyclin A, E, D, CDK 4 and alpha-tubulin. **D.** PC3 or DU145 cells were plated in media containing 10% FBS and treated with increasing concentrations G1T28 (10^{-10}-$10^{-4.3}$ M) and measured for cell viability by Hoechst staining after 5-7 days of treatment. Data are representative of at least 3 independent experiments.

Supplemental Figure 2. Analysis of pharmacodynamics endpoints indicates on-target activity in 22Rv1 tumors. A. Levels of AR, AR-V7, phospho-Rb, and total Rb were evaluated by immunoblot in 22Rv1 tumors harvested from two mice per group euthanized after 10 days of treatment from the efficacy study in Figure 2. **B.** Chemical structure of CDK4/6 inhibitor G1T38.

Supplemental Figure 3. G1T38 and G128 have similar *in vitro* efficacy. A. LNCaP, VCaP and 22Rv1 cells were plated in media containing 10% FBS and increasing concentrations of G1T28 or G1T38 (10^{-9}-10^{-6} M) for 24 hours. Following treatment cells were fixed, permeabilized and stained for phosphorylated Rb (pRb) or total Rb. Cells were imaged using the LI-COR Clx and dose response curves were generated from densitometry of the phospho/total Rb expression using LI-COR Image Studio software. **B.** LNCaP, VCaP, or 22Rv1 cells were plated in media containing 10% FBS and treated with increasing concentrations of G1T28 and G1T38 (10^{-9}-$10^{-5.5}$ M) and measured for cell viability by Hoescht staining after 5-7 days of treatment. **C.** PC3 or DU145 cells were plated in media containing 10% FBS and treated with increasing concentrations of G1T28 and G1T38 (10^{-9}-$10^{-5.5}$ M) and measured for cell viability by Hoechst staining after 5-7 days of treatment. **A-C.** Data are representative of at least 3 independent experiments. **D.** Individual body weight was measured for 28 days in mice bearing 22Rv1 xenografts treated with Vehicle, G1T38 (100mg/kg, p.o, q.d.), or docetaxel (20 mg/kg, i.p., q.w.).

Supplemental Figure 4. G1T28 inhibits anti-androgen stimulated prostate cancer cell growth. A. LnCAP cells stably expressing a control plasmid pQC-XIP (XIP), wild type AR (XIP-AR) or AR with the activating F876L point mutation (XIP-AR F876L) were plated in phenol red free media containing 10% charcoal stripped serum and treated with increasing concentrations of G1T28, or the anti-androgens casodex or enzalutamide (10^{-9}-10^{-4} M). **B.** LnCAP-AR or LnCAP-AR F876L cells were treated with 25μM or 20μM of casodex or enzalutamide respectively in the presence of increasing concentrations of G1T28. Cell viability was measured by Hoechst staining after 5-7 days of treatment. Data are representative of at least 3 independent experiments.