

Figure S1. The DHT does not significantly affect p27 mRNA levels. CWR22R3 cells were cultured in CDS-serum medium and treated with 10nM DHT or carrier ethanol (EthOH) for the indicated time. The total RNAs were collected and p27 message level was measured by real time RT-PCR and presented as relative level compared to carrier treatment. Error bar, mean±SEM (n=3).

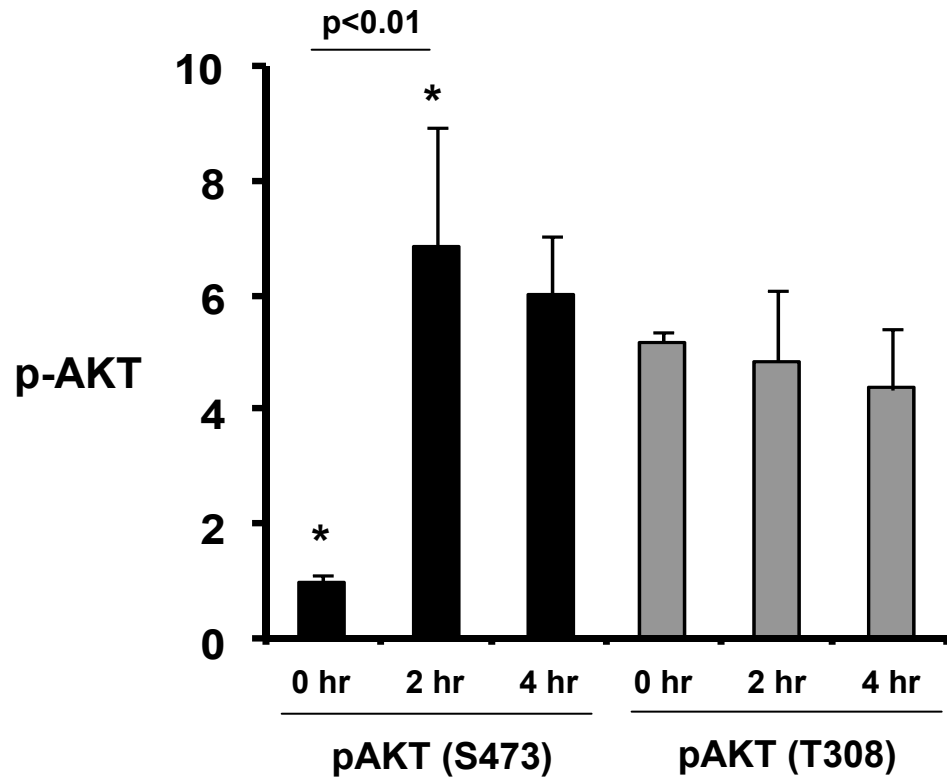


Figure S2. DHT stimulated AKT S473 phosphorylation.

CWR22R3 cells cultured in CDS-serum medium were treated with 10 nM of DHT for the indicated time period and blotted for phospho-S473, -T308 and total AKT. The immunoblot bands were quantified by ImageJ and normalized against total AKT; (n=3). (*: Student T test, p<0.01). A rabbit polyclonal phospho-AKT (T308) was used.

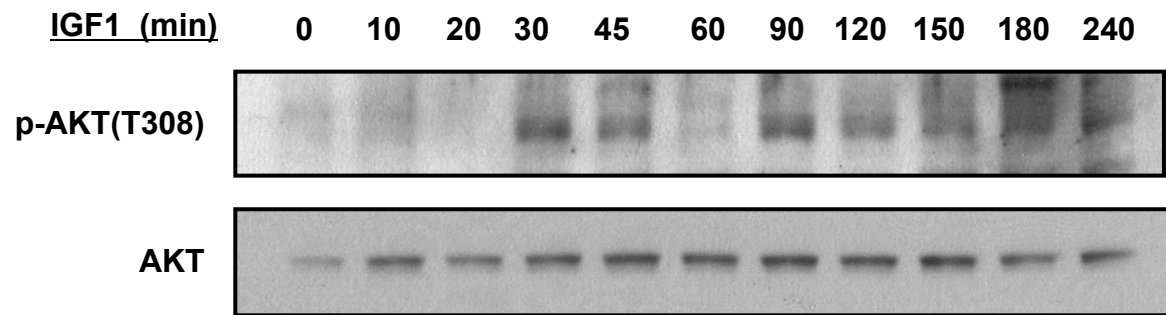


Figure S3. IGF1 stimulates AKT T308 phosphorylation. CWR22R3 cells cultured in CDS medium were treated with 20 ng/ml of IGF1 for the indicated time and blotted for phospho-T308 and total AKT. A rabbit polyclonal phospho-AKT (T308) was used.

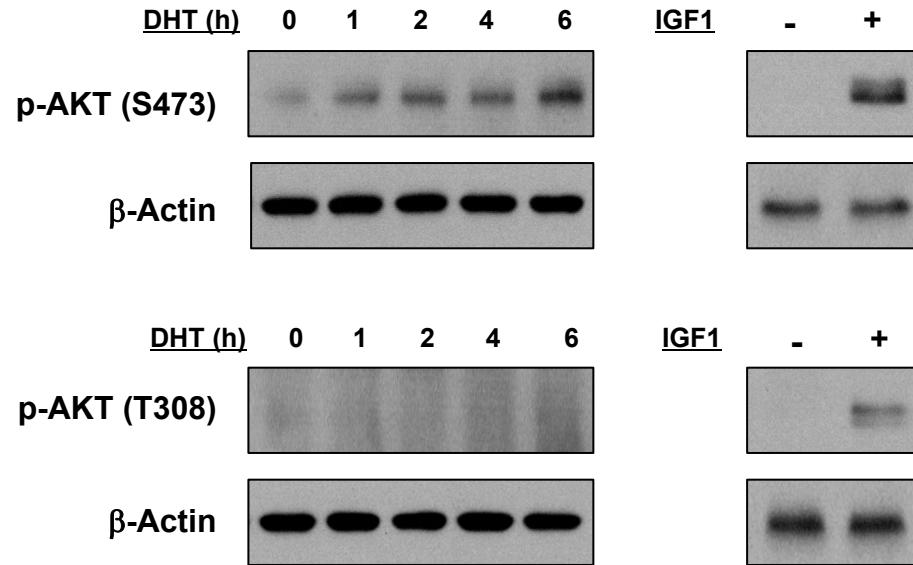


Figure S4. DHT induced AKT S473 phosphorylation. CWR22R3 cells were cultured in serum-free medium for 24 hours, followed by treatments with 10nM DHT from 0-6 hours; or by treatments with 20ng/ml IGF1 for 0 (-) or 15 (+) minutes. A rabbit monoclonal phospho-AKT (T308) was used for T308 phosphorylation. The β-Actin was blotted for protein loading control.

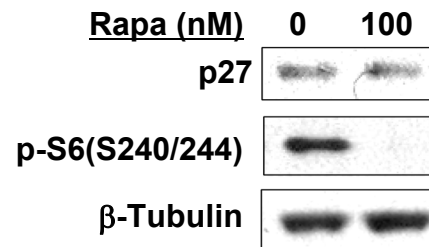


Figure S5 Rapamycin does not affect basal p27 levels. CWR22R3 cells were treated with rapamycin at the indicated dose for 8 hours and p27 and p-S6 were blotted.

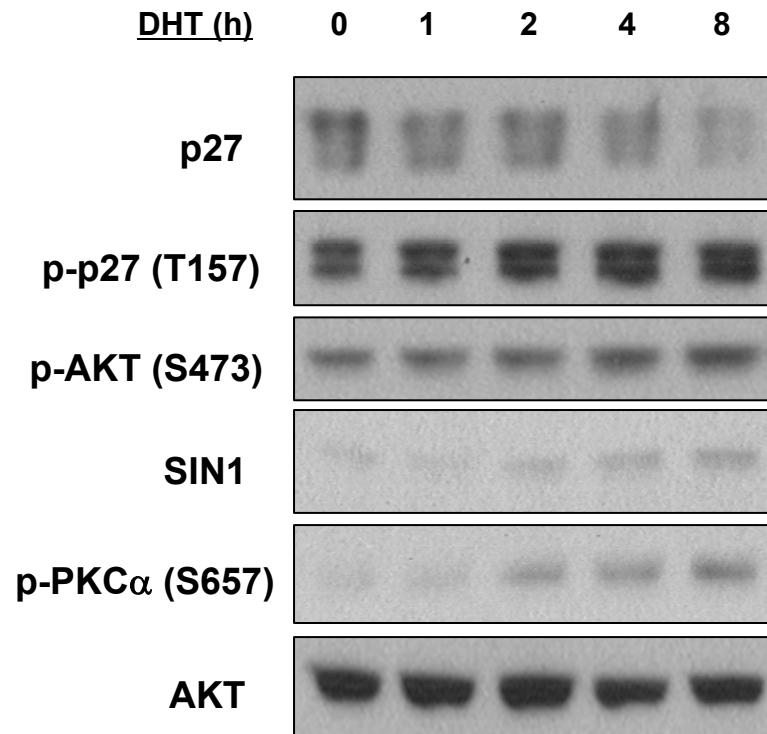


Figure S6. DHT induced TORC2 activation and p27 degradation in LNCaP cells. LNCaP cells cultured in medium with CDS serum for 72 hours were treated with DHT (10 nM) for 0 to 8 hours and the cell lysates were immunoblotted. Total AKT was blotted for protein loading control.

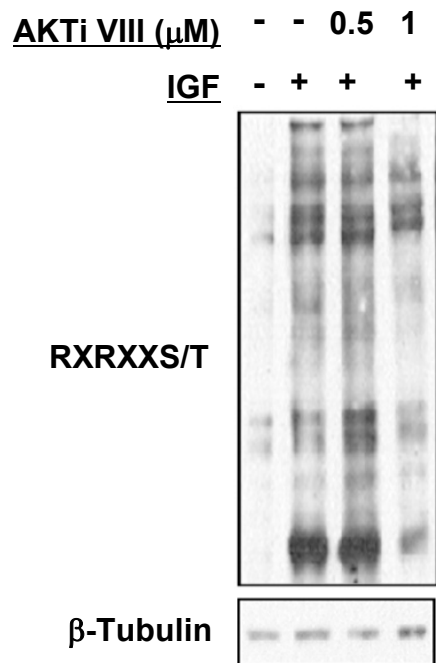


Figure S7. A dose-dependent suppression of AKT activity by an AKT inhibitor (AKTi VIII). CWR22R3 cells were treated with AKT inhibitor (AKTi VIII) for 2 hours at indicated concentrations. At the end of incubation, the cells were pulsed with IGF1 (20 ng/ml) for 15 minutes.

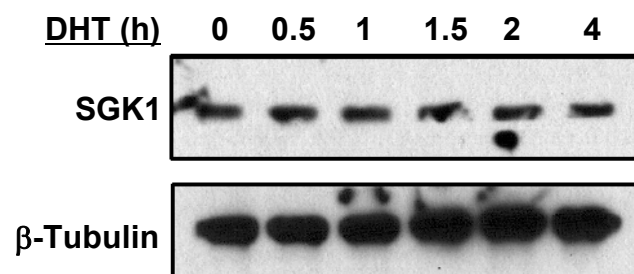


Figure S8. DHT does not acutely affect SGK1 protein levels.
CWR22R3 cells were treated with 10nM DHT for the indicated time and blotted.