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**Supplementary Figure S5. CYP27A1/27HC axis inhibits growth of PC cells via depletion of cellular cholesterol**

**A)** Cholesterol was measured using a targeted LC-MS/MS method from the pellets of LNCaP or 22RV1 cells overexpressing CYP27A1 or GAL4 control following 7 days of doxycycline treatment (25 ng/ml). Experiment was repeated three times and error bars represent standard error of the mean (\*p < 0.05, unpaired t-test). **B)** Cholesterol was measured from four representative tumors harvested at sacrifice from each experimental arm (GAL4 or CYP27A1) of the animal study described in Figure 2. Error bars represent the standard error of the mean and \*p<0.05 was considered a significant variation (unpaired t-test)). **C)** LNCaP or 22RV1 cells were treated with vehicle, 5 μM of 27HC, 10 μM of cholesterol, or a combination of 27HC and cholesterol. Seventy-two hours later the cell lysates were harvested and immunoblot analysis was performed to analyze expression levels of C-PARP and GAPDH. **D)** LNCaP or 22RV1 cells were treated with vehicle, 5 or 10 μM 27HC in the presence or absence of 10 μM cholesterol. Three days later the cells were harvested and stained with Sytox and Annexin V. The percentage of cells that stained for either of the above dyes was analyzed using FACS. Annexin V staining is represented on the X-axis while Sytox staining is represented on the Y-axis. Flow cytometry data shown is a representative of three independent experiments. **E)** LNCaP or 22RV1 cells were plated in 96-well plates. The cells were then treated with LDL (0.25 mg/ml) added to full serum containing media or full serum media alone. Two days later the cells were treated with the indicated doses of 27HC or vehicle control. Seven days later the cells were harvested and the final DNA content determined via staining with the DNA dye Hoechst 33258.