Supplemental Figure 1. AZD5363 induces apoptosis in LNCaP prostate tumor cell line. A, LNCaP were treated for 48h with AZD5363 at indicated doses. The proportion of cells in subG1, G0-G1, S, or G2-M was determined by propidium iodide staining. B, LNCaP cells were treated with 1µM AZD5363 at indicated time points. Protein extracts were analyzed for interest proteins involved in apoptosis pathway.

Supplemental Figure 2. A, AZD5363 fails to induce apoptosis in PC-3 and DU145 cells. PC-3 and DU145 cells were treated with AZD5363 (100µM for 48H), staurosporine (1µM for 16H) and MG132 (20µM for 16H). Total proteins were extracted and analyzed by western blot using cleaved PARP and cleaved caspase-3, vinculin was used as a loading control. B, mTOR inhibitor (Rapamycin) induces autophagy. PC-3 cells were treated with either rapamycin (100nM for 48H) or chloroquine (10µM for 48H) or in combination. Total protein extracts were analyzed by western blotting for LC3-I, LC3-II, p-Akt and its downstream effector pS6 kinase, vinculin was used as the loading control.

Supplemental Figure 3. Targeting autophagy enhances PI-103 activity to induce apoptosis in PC-3 tumor prostate cells. PC3 cells were treated with 250 nM of PI-103 alone or in combination with 20µM chloroquine (CHQ) for 48h. Cell proliferation was determined by crystal violet (A). Protein extracts were analyzed by western blotting for LC3, Akt, p-Akt, p-S6 and PARP (B).

Supplemental Figure 4. AZD5363 doesn’t affect RIP kinase and ROS. A, PC-3 cells were treated with 10 µM of AZD5363, rapamycin (50 nM) or PI-103 (0.5, 1 µM) for 48 hours. Total proteins were extracted and analyzed by western blot using RIPK, actin was used as a loading control. B, PC3 cells were treated with AZD5363 (10µM), Rapamycin (50nM) or PI-103(0.5µM)
for 4 hours respectively followed by incubation with 10 µM 2’-7-dichlorodihydrofluorescence diacetate (DCF-DA, Molecular Probes, Invitrogen) for 10 min. Ten thousands live cells were collected and fluorescent signaling was examined by flow-cytometry (BD FACSCanto™ II).