Supplementary Figure 3: AZD5363 treatment activates the AR and receptor tyrosine kinases pathways:

LNCaP cells were treated with 5µM of AZD5363 (A) or 1µM of rapamycin (B) for 48 hours, total protein were extracted and western blot was performed using AR, PSA, pS6/S6, pERK/ERK, PHLPP, vinculin was used as a loading control. C, inhibition of AKT by siRNA results in activation of PSA. Protein expression levels of AKT and PSA were measured after 48hr treatment with scrambled control siRNA or siAKT. Total proteins were extracted and western blot was performed using AKT, PSA antibodies and vinculin was used as a loading control. D, AZD5363 induces a feed-forward loop activating receptor tyrosine kinases. LNCaP cells were treated with AZD5363 for 24 hours. Total proteins were extracted and western blots were performed using pEGFR/EGFR, pIGFR/IGFR, vinculin was used as a loading control.