Supplementary Figure Legends

Supplementary Fig. S1. AZD5363 inhibits S6 phosphorylation in TSC1 -/-, TSC2 low-expressing RT4 bladder cancer cells. Phosphorylated and total protein levels of S6, P70S6K, and total GAPDH were measured by Western blotting, following exposure to either the allosteric AKT inhibitor MK-2206 or AZD5363 *in vitro*.

Supplementary Fig. S2. Correlation of AZD5363 activity in cell lines from different tumor types with PIK3CA and PTEN mutation status. Inhibition of cell proliferation, measured by MTS assay (vertical axis, log scale) was correlated with genetic status, as defined in the figure. The cut off that defines "sensitive" (GI50 < 3 μM; solid line) and "highly sensitive" (GI50 < 1 μM; dashed line), and the position of the highly sensitive BT474c breast, LNCaP and PC346C-Flut1 prostate cancer cell lines, is shown.

Supplementary Fig. S3. Correlation of AZD5363 activity in cell lines from different tumor types with RAS, PIK3CA and PTEN status. Inhibition of cell proliferation, measured by MTS assay (vertical axis, log scale) was correlated with genetic status, as defined in the figure. The cut off that defines "sensitive" (GI50 < 3 μM; solid line) and "highly sensitive" (GI50 < 1 μM; dashed line), and the position of the highly sensitive BT474c breast, LNCaP and PC346C-Flut1 prostate cancer cell lines, is shown.

Supplementary Fig. S4. AZD5363 has a predominantly anti-proliferative mechanism of action but induces cell death in BT474c, LNCaP and PC346C-Flut1 cells *in vitro*, and in BT474c xenografts following a high, intermittent dosing schedule *in vivo*. A: Inhibition of cell proliferation was measured using MTS and Sytox Green assays. The Sytox Green assay also gives a read-out of % cell death. Induction

of cell death in the BT474c cell line was confirmed by monitoring cleaved caspase 3 and cleaved PARP by western blotting. B: Comparison of the effect of high, intermittent and lower continuous dosing schedules of AZD5363 on BT474c xenograft growth. Cleaved caspase 3 and Ki67 immunostaining was carried out on xenografts at the stated time-points after the final dose of compound, following 4 days of treatment.

Supplementary Fig. S5. Quantification of cleaved caspase 3 activity in HCC-1187 xenografts following chronic dosing with 5 mg/kg once weekly docetaxel (taxotere), 150 mg/kg bid AZD5363, or a combination of these two agents. Cleaved caspase 3 was measured by immunocytochemistry in xenografts at 4 h after the final dose of compound(s).