Supplementary Figure 1. PC-3 overexpressing-SPL cell lines exhibit a lower rate of cell viability associated with enhanced basal apoptosis

A, the percentage of viable cells was determined by the trypan blue dye exclusion method. Adherent cells were detached by using 0.05% trypsin-EDTA and stained with trypan blue, which stains dead cells. Floating cells were recovered, centrifuged and resuspended in PBS 1X before counting. The viable cells were quantified in a Neubauer chamber. The data represents an average of three independent experiments.

B, cell lysates were analyzed for processed caspase-3 and PARP expression. Equal loading of protein was monitored using antibody to α-tubulin. Western blots are representative of three independent experiments.

Supplementary Figure 2. The modulation of S1P content in C4-2B prostate cancer cells alters sensitivity to irradiation

C4-2B cells were transfected with 75 nM siScramble, 75 nM siSPL1b or 33 nM siSphK1 for 72h (A) or treated with 5 µM exogenous S1P for 30 min then removed (B). Cells were then irradiated from 2 to 6 Gy and survival clones were counted 6 days later. Data are expressed as the percentage of survival fraction compared to non-irradiated cells. Points, mean of at least 4 independent experiments; bars, SEM. The two-tailed P values between the means of siScramble and siSphK1 or siSPL1b treated cells (A) or the means of untreated and S1P-treated cells (B) are: *, P < 0.05; **, P < 0.01; ***, P < 0.001. C, cell lysates from C4-2B cells treated with siScramble, siSPL1b or siSphK1 before irradiation (2 Gy) and recovered at different times were analyzed for γ-H2AX expression. Equal loading of protein was monitored using antibody to α-tubulin. Similar results were obtained in three independent experiments.
Supplementary Figure 3. The silencing of SphK2 activity does not sensitize to docetaxel nor to radiotherapy in PC-3 and C4-2B cell lines

A, PC-3 and C4-2B cells were untransfected or transfected with 50 nM of ON-TARGETplus SMARTpool siRNA against SphK2 (Hait et al., Science, 2009) or 50 nM scrambled siRNA (siScramble) for 72h. Cell lysates were assayed for SphK2 activity and expression by western blot analysis (Santa Cruz, SC-2270). Equal loading of protein was monitored using antibody to α-tubulin. Similar results were obtained in three independent experiments. Columns, mean of 3 independent experiments; bars, SEM. The two-tailed $P$ values between the means of untreated, and siSphK2 or siScramble treated cells are: ns, not significant; **, $P < 0.01$; ***, $P < 0.001$.

B, PC-3 (left panel) and C4-2B (right panel) cells were transfected with 50 nM siScramble or 50 nM of ON-TARGETplus SMARTpool siRNA against SphK2 for 72h. Cells were then irradiated from 2 to 6 Gy and survival clones were counted 6 days later. Data are expressed as the percentage of survival fraction compared to non-irradiated cells. Points, mean of 3 independent experiments; bars, SEM. The two-tailed $P$ values between the means of siScramble and siSphK2 treated cells are not significant.

C, PC-3 (left panel) and C4-2B (right panel) cells were transfected with 50 nM siScramble or 50 nM of ON-TARGETplus SMARTpool siRNA against SphK2 for 72h. Cells were then treated in presence or not of 20 nM docetaxel for 72h, and cell viability was assessed by MTT. Data are expressed as the percentage of cell viability compared to untreated cells. Points, mean of 3 independent experiments; bars, SEM. The two-tailed $P$ values between the means of siScramble and siSphK2 treated cells are not significant.
Supplementary Figure 4. S1P degradation products do not sensitize to radiotherapy or chemotherapy in PC-3 and C4-2B prostate cancer cells

PC-3 and C4-2B prostate cancer cells were treated with increasing concentrations of hexadecanol or palmitic acid. A cell viability was assessed in PC-3 (left panel) and C4-2B (right panel) by MTT assay after 48h of treatment. Data are expressed as the percentage of cell viability compared to untreated cells. Columns, mean of 3 independent experiments; bars, SEM. The two-tailed P values between the means are: ns, not significant; * P < 0.05; **, P < 0.01. Hexadecanol or palmitic treatment was combined with increasing doses of irradiation (B) or docetaxel (C) in PC-3 (left panel) and C4-2B cells (right panel). Data are expressed as the percentage of survival fraction compared to non-irradiated cells or as the percentage of cell viability compared to docetaxel untreated cells. Points, mean of at least 3 independent experiments; bars, SEM. The two-tailed P values between the means of untreated cells and the other experimental conditions are: ns, not significant.