Supplementary Figure Legends

Supplementary Fig. 1 MEK5 protein expression in human prostate cancer cell lines. MEK5 protein abundance in a panel of normal (PrEC), immortalized (EP156T), and cancer (LNCaP, PC3, DU145) human prostate cell lines.

Supplementary Fig. 2 *MEK5* siRNA and shRNA efficiency. **a, b** MEK5 protein levels in DU145 (a) and PC3 (b) cells transiently transfected with *Luciferase* (DL or PL) or four (DU145; D76, D78, D10, D20), or two (PC3; P76, P78) independent *MEK5* siRNAs. **c, d** Stable clones of PC3 (#12, #22) (c) and DU145 (#5, #7, #9) (d) expressing *MEK5* shRNA or a scrambled (shControl) shRNA. **e** Ectopic expression of MEK5-pcDNA3 construct in PC3 stably expressing MEK5 shRNA (clone #12). Lysates were immunoblotted with anti-MEK5 and either anti-α-tubulin or anti-β-actin (loading controls).

Supplementary Fig. 3 (Related to Fig. 1g) Equal number of DU145 cells stably expressing control or MEK5 (clone #9) were exposed to 4 Gy γ-rays or were sham irradiated. Cell proliferation was measured 5 days post-irradiation and expressed as percent proliferation relative to unirradiated cells. Shown mean ± S.D. (n = 3). P value was calculated by Student's t-test.

Supplementary Fig. 4 MEK5 downregulation does not influence cell cycle distribution within 48h of irradiation. **a** Cell cycle profile of DU145 (*left*) and PC3 (*right*) cells transiently expressing *Luciferase* or *MEK5* siRNA exposed to 3 Gy γ-rays or were sham irradiated (UI). **b** quantitation of cell cycle distribution of PC3 and DU145 cells stably expressing scrambled (shControl) or

MEK5 (PC3 clones #12 and #22; DU145 clone #9) shRNA. % G1, S, and G2/M phases were quantitated by FlowJo software. Shown mean ± S.E.M (n = 3).

Supplementary Fig. 5 (Related to Fig. 2) Additional experiments (expt. 2 and expt. 3) with PC3 cells expressing *shControl* or *shMEK5* exposed to irradiation and immunoblotted for DNA-PKcs (S2056), ATM (S1981) phosphorylation. Blots were re-probed with antibodies against total DNA-PKcs and ATM (expt.3). UI: unirradiated.

Supplementary Fig. 6 EP156T cells (**a)** or PC3 and LNCaP (**b**) were transiently transfected with *Luciferase* or *MEK5* (#78) siRNA and 6 days later they were exposed to 3 (PC3, EP156T) or 2 (LNCaP) Gy of γ-rays. Cells were lysed at various times and immunoblotted with the indicated antibodies. **c** LNCaP cell proliferation 6 days post-irradiation was measured by staining cells with crystal violet and recording absorbance at 595 nm. Mean ± S.D. (n =3) expressed as percent change compared with control unirradiated LNCaP cells. UI: unirradiated. IR: irradiated. NS: not significant.

Supplementary Fig. 7 (Related to Fig. 3) PC3 (*shControl*, *shMEK5* #12) (**a**) or DU145 (*shControl*, *shMEK5*#9) (**b**) cells were serum-starved for 24h, then treated with etoposide (1 μM, 16h). The drug was removed, fresh medium (no serum) was added and cells were incubated for various times. Lysates were immunoblotted with the indicated antibodies. UT: untreated.

Supplementary Fig. 8 (Related to Fig. 4) PC3 cells transiently expressing *Luciferase* (siLUC) or *MEK5* siRNA were irradiated with 3 Gy and at the indicated times they were fixed and stained for

γH2AX, 53BP1, and 4', 6-diamidino-2-phenylindole (DAPI; DNA). **a** Representative images and **b** western blot analysis of MEK5 protein levels in *LUC* and *MEK5* siRNA cells. **c** quantitation of number of γH2AX foci per cell. **d** quantitation of number of 53BP1 foci per cell. Shown mean ± S.D. (n =3). * $p < 0.001$, calculated by Student's t-test. UI: unirradiated.

Supplementary Fig. 9 Cell proliferation. PC3 (shControl, shMEK5 clone#12) were seeded in 12-well plates (5,000 cells/well). Cells were trypsinized and counted with a hemocytometer at days 0, 2, 3, and 6. Mean ± S.D. (n = 3).

Supplementary Fig. 10 Radiation plan dosimetry and dose-volume histograms. **a** Representative images in coronal, axial and sagittal orientation of tumor-bearing mouse with radiation target volumes (tumor, red; tumor isocenter, cyan) contoured on cone-beam computed tomography images imported into MuriPlan software (Xstrahl). **b, c** Representative dose-volume histogram (DVH) and corresponding dosimetry to tumor (mean, minimum and maximum radiation dose in cGy) for tumor radiotherapy treatment plans.