**Supplementary Materials & Methods**

**Generation of Adenoviral and Lentiviral Vectors**

The RNAi Consortium (TRC) pLKO.1 lentiviral vectors containing shRNA targeting Stat5a/b (shStat5a/b), Jak2 (shJak2) and shRad51 (Thermo Fisher Scientific), and non-target control (shCtrl) (Dharmacon, Inc.). Second-generation VSV-G pseudotyped high-titer lentiviruses were generated by transient co-transfection of 293FT cells, with a three plasmid combination of pLKO.1 lentiviral vector containing shRNA of interest, pHR'8.2ΔR packaging plasmid, and pCMV-VSV-G envelope plasmid.

PcDNA-CMV-WTStat5b (AdWTStat5b), pcDNA-CMV-DNStat5a/b (AdDNStat5a/b), pMod-DNR-CAStat5a (S710F), pMod-DNR-CAStat5b (S715F), and pMod-DNR-GFP were cloned into adenoviral vectors using the BD Adeno-XTM Expression System 2 BD Biosciences Clontech, Palo Alto, CA) per the manufacturer’s protocol. Purified recombinant adenoviruses were linearized by PacI digestion and transfected to QBI-293A cells (Qbiogene, Carlsbad, CA) to produce infectious adenoviruses. The viral stocks were expanded in large-scale cultures, purified using double cesium chloride gradient centrifugation and titrated by a standard plaque assay method in QBI-293A cells, as per the manufacturer’s instructions (27,29,35).

**SiRNA and Stat5a/b Antisense Transfection**

PC cells were transfected with Stat5a siRNA, Stat5b siRNA or non-target control siRNA (Dharmacon, Inc.) using Lipofectamine 2000 (Invitrogen). Alternatively, cells were transfected with 900 pM Stat5a/b antisense (AS) or mismatch control (Ctrl) oligodeoxynucleotides (ODN; IONIS Pharmaceuticals) (34)using jetPEITM reagent (QBiogene Inc.).

**Immunoprecipitation and Immunoblotting**

Cells were solubilized in the lysis buffer [10 mM Tris-HCl (pH 7.5), 5 mM EDTA, 50 mM NaCl, 30 mM sodium pyrophosphate, 50 mM sodium fluoride, 1 mM sodium orthovanadate, 1% Triton X-100, 1 mM phenylmethylsulphonylfluoride, 5 µg/ml aprotinin, 1 µg/ml pepstatin A and 2 µg/ml leupeptin]. Stat5a, Stat5b pAbs (Millipore), Jak2 pAb (Millipore), BRCA1 pAb (Santa Cruz) and BRCA2 pAb (AbCam) were immunoprecipitated from cell lysates followed by incubation with protein A-Sepharose beads (GE Healthcare). The cell lysates and immunoprecipitates were separated on 4-12% SDS-PAGE (Life Technologies) and transferred electrophoretically to polyvinylidene fluoride membrane (Millipore). For immunoblotting, blocking buffer used was Tris-Buffered Saline and Tween 20 (TBST, 0.15 M NaCl; 0.1% Tween 20; 50 mM Tris, pH 8.0) with 3% Bovine Serum Albumin (BSA). Primary antibodies used for immunoblotting were: anti-pYStat5a/b (Y694/Y699 mAb; BD Biosciences, Inc.), anti-Stat5a/b (mAb; BD Biosciences, Inc.), anti-Rad51 (pAb; Santa Cruz), anti-BRCA1 (Cell Signaling) and anti BRCA2 (mAb; Santa Cruz) or anti-actin (pAb; Sigma). The immunoreaction was detected by horseradish peroxidase-conjugated goat anti-mouse secondary antibodies (1:2000; BD Biosciences).

**Immunofluorescence Staining**

CWR22Rv1 or CWR22Pc cells on chamber slides (Fisher Scientific) were treated with 3.1 or 6.3 µM IST5-002 or DMSO control for 48 h. Cells were irradiated with 0 or 3 Gy for 4 h and immunostained for γH2AX. Briefly, irradiated cells were fixed and incubated with anti-γH2AX mAb (Millipore). Antigen-antibody complexes were visualized with Texas red-conjugated anti-mouse secondary antibody (Vector Labs). The images were captured using Zeiss LSM510 laser-scanning microscope equipped with 40x objective.

**Immunohistochemistry and Scoring of Cell Viability and Immunostainings**

Immunohistochemical staining of xenograft tumors and patient-derived PCs cultured *ex vivo* in tumor explant cultures was performed as described previously (26,27,29,35-37). Formalin-fixed sections were immunostained for Rad51 (mAb; Abcam), γH2AX (mAb; Millipore) and Stat5a/b (mAb; Santa Cruz Biotechnology). Antigen retrieval for all three Abs was performed using citric acid buffer (pH 6.0) (Stat5), followed by incubation with primary antibody and horseradish peroxidase–conjugated IgG secondary antibody (30 minutes; Dako). Expression of nuclear active Stat5a/b, Rad51, and γH2AX are expressed as percentage of positively stained cells, as previously described (29,35-37). Briefly, protein levels were scored blindly (V.U., K.M., and M.T.N.) and presented as an average from three separate views per tissue sample using the H-score method (50), an assessment of both intensity of staining and percentage of stained cells.

***Ex vivo* 3D Tumor Explant Cultures of Patient-Derived PCs.** PC specimens were obtained from patients with organ-confined PC undergoing radical prostatectomy and bilateral iliac lymphadenectomy Table 1). The Medical College of Wisconsin Institutional Review Board found this research to be in compliance with federal regulations governing research on de-identified specimens and/or clinical data [45 CFR 46102(f)]. Briefly, PC tissues were cut into approximately 1-mm3 pieces in growth medium, as previously described (26,35-37,42), transferred onto matrix-covered grids in petri dishes, and cultured at 37°C in a mixture of oxygen, carbon dioxide, and nitrogen (40:5:55) for seven days in the presence or absence of IST5-002 (25 µM) or AZD1480 (25 µM) , and the growth medium was changed every other day.

**Rad51 Proteasomal Degradation Analysis**

Cells were treated with 10 µM MG132 (Calbiochem) for 6 h and cycloheximide (35 µM), where indicated, before harvest and immunoblotting, as previously described.

**Quantitative real-time RT-PCR**

Total RNA was reverse-transcribed using SuperScript III First-Strand Synthesis System (Invitrogen). Quantitative PCR was carried out using forward (qRad51F: 5’-gctgcggaccgagtaatg-3’) and reverse (qRad51R: 5’-ccagcttcttccaatttcttcac-3’) primers and **HotStart-IT SYBR Green** One-Step qRT-PCR Master Mix (Affymetrix, Santa Clara). Fold-change in expression levels (using glyceraldehyde-3-phosphate dehydrogenase as control) was determined by a comparative CT method using the formula 2-ΔΔCT; where CT is the threshold cycle of amplification.

**Statistical Analyses**

For comet assay, mixed effects linear regression was used to model “olive tail moment” as a function of time and group. A heterogeneous compound symmetry covariance structure with group-specific variances was assumed. Groups were compared at 1 hour and at 24 hours after IR. When there was a difference in groups, post-hoc pairwise comparisons were performed comparing AdDNStat5a/b to the other three groups. P-values for post-hoc comparisons were adjusted using the Bonferroni method. For Rad51 foci formation assay,Pearson’s chi-square test was used to compare control siRNA to Stat5a/b siRNA with respect to the proportion of cells with Rad51 foci formation and quantitative analysis of Rad51 mRNA expression. For HR DNA repair, analysis of variance was used to compare groups with respect to mean levels of fold changes. Pairwise comparisons were Bonferroni adjusted. For pulsed-field gel electrophoresis analyses, repeated measures analysis of variance was used to model log-transformed relative ratios dsDNA breaks at times 10 through 60. Fixed effects were included for time, group, and their interaction. A first-order auto-regressive covariance structure was assumed for residual errors. In the clonogenic survival assays, the relationship between the survival fraction and radiation dose level was modeled using linear regression. Survival fractions were logit transformed (i.e., log(sf/[1-sf])) to better meet assumptions of normality of residuals. Groups were compared with respect to the survival fraction at different radiation doses. In addition, the slope for each group was estimated as a measure of the effect of each additional Gy of radiation in reduction of the logit survival fraction. For the analyses of tumor growth, log-10 transformed tumor volumes were modeled using mixed effects linear regression. A first-order autoregressive covariance structure was assumed to account for correlation among repeated measurements from the same animal. The model fit a linear curve in time for each group with a common intercept to the log-10 transformed volumes. This slope was expressed in terms of the percentage change in volume per day and was expressed as tumor doubling time. Groups were compared with respect to the average slopes. A test of any group differences in slopes was performed followed by pairwise comparisons of slopes, and the analysis was performed using SAS 9.4.

With respect to cell viability, Stat5, Rad51 and γH2AX-positive cells, linear mixed effects models with empirical standard errors were used to compare groups. Cell viability was logit transformed (i.e., log (y/(1-y)) prior to analysis to better meet assumptions of normality of residual errors. A random intercept term accounted for correlation among repeated measurements of the same slide. Post-hoc pairwise comparisons were performed between each treatment group and vehicle, and p-values for these comparisons were adjusted using Dunnett’s method.