Supplementary Information to accompany Kounatidou et al.

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Figure legends

Supplementary Figure S1. Validating CRISPR knock-in strategy in CWR22Rv1 cells. a. CWR22Rv1 cells were transiently transfected with either Cas9/gRNA_1- or Cas9/gRNA_2-encoding plasmids and immunofluorescence images were taken to demonstrate successful expression of the plasmid-derived GFP marker (left panel). Resultant SURVEYOR endonuclease assay of CRISPR target amplicon of the AR gene exon 5 locus from Cas9/gRNA_1- and Cas9/gRNA_2-transfected CWR22Rv1 cells (* indicates successful cleavage of hetero-duplexes generated as a consequence of mixing equal amounts wild-type and CRISPR-modified amplicons). b. TIDE analysis of resultant sequencing data derived from Cas9/gRNA_1- and Cas9/gRNA_2-transfected cells (PAM sites for both CRISPR complexes are shown at top of right panel). c. Diagrammatic representation of donor template used to knock-in stop codon (TAA) into exon 5 of the AR gene. TTAA sequence represents an Mse I restriction enzyme site to enable restriction fragment length polymorphism (RFLP) analysis. F- and R-labelled arrows indicate primers used to amplify DNA from clonal cell populations to identify incorporation of donor template into the host genome. d. Successful detection of donor template incorporation into CWR22Rv1 cells as indicated by the generation of two DNA fragments by Mse I-mediated RFLP to form the derivative cell line CWR22Rv1-AR-EK.

Supplementary Figure S2. Validating loss of FL-AR in CWR22Rv1-AR-EK cells. CWR22Rv1- and CWR22Rv1-AR-EK-derived lysates were subject to immunoblotting using four distinct AR N-terminal-targeting antibodies: AR N20 (Santa Cruz Biotechnology - discontinued), AR-BD (BD Pharmingen), AR ab74272 (Abcam) and AR-441 (Santa Cruz Biotechnology). Short and long exposure were performed to enable detection of FL-AR in CWR22Rv1 parental cells. α-tubulin was used as loading control.
**Supplementary Figure S3.** Validating genomic integrity of CWR22Rv1-AR-EK cells. 

- **a.** Bright field images of CWR22Rv1 and CWR22Rv1-AR-EK cells were taken at a 10x magnification to demonstrate similar morphology of the two cell lines. 
- **b.** CWR22Rv1 and CWR22Rv1-AR-EK share equivalent genomic SNP markers as determined using conventional cell line authentication. 
- **c.** Predicted potential CRISPR off-target sites were amplified using specific primers and resultant amplicons were analysed by TIDE. Sequencing chromatograms of the three highest ranked off-target loci within COL1A2, KMT2B and SLC7A8 genes are shown for parental and CWR22Rv1-AR-EK cells (left panel) and analysed by TIDE (right panel).

**Supplementary Figure S4.** AR-V depletion in CWR22Rv1-AR-EK down-regulates androgenic gene expression. CWR22Rv1-AR-EK cells grown in steroid-depleted media were subject to control (siScr) or AR-V (siAR-V) depletion for 48 hours with either vehicle, 10 nM DHT or 10 µM enzalutamide (Enz) treatment for the final 24 hours before quantitative RT-PCR to assess UBE2C, ATAD2 and KLK2 expression. Data represents the average of three independent experiments +/- SD.

**Supplementary Figure S5.** AR target gene expression in CWR22Rv1-AR-EK is driven exclusively by AR-Vs. 

- **a.** CWR22Rv1-AR-EK cells grown in steroid-depleted media were subject to transfection with control (siScr) or FL-AR-targeting siRNAs (siARex4 and siARex7) for 48 hours with either vehicle or 10 nM DHT treatment for the final 24 hours before quantitative RT-PCR to assess PSA, KLK2 and UBE2C expression. Data represents the average of three independent experiments +/- SD. 
- **b.** Cell lysates harvested from the parallel experiment were subject to western blot analysis using anti-AR and α-tubulin antibodies to demonstrate that unlike siARex1, both siARex4 and siARex7 have no impact on AR-V levels in CWR22Rv1-AR-EK cells (left panel). Using CWR22Rv1 parental cells, siARex4 and siARex7 were able to successfully deplete FL-AR levels (right panel).
Supplementary Figure S6. AR-Vs associate with AR-target genes in CWR22Rv1-AR-EK cells. CWR22Rv1-AR-EK cells were subject to control (siScr) or AR (siARex1) knockdown for 48 hours before ChIP experiments, incorporating either N-terminal AR-binding or control (IgG) antibodies. Data represents the average of three independent experiments +/- SD (** represents p<0.01 as determined using one-way ANOVA).

Supplementary Figure S7. Validating samples for RNA sequencing analysis. a. Triplicate CWR22Rv1-AR-EK samples transfected with either control (siScr) or AR-V-targeting (siARex1) siRNAs were subject to quantitative RT-PCR and immunoblotting to assess effect on AR-target gene expression (left panel) and validate AR-V depletion (right panel). Data represents the average of three independent experiments +/- SD (** represents p<0.01 as determined using a two-tailed student T-test). b. MA plot of RNA sequencing data from three experimental replicates showing statistically significant gene expression changes (p<0.01) above and below the 0 y-intercept representing up- and down-regulated genes, respectively, shown in red.

Supplementary Figure S8. Reduced CWR22Rv1-AR-EK cell growth in response to AR-V depletion. CWR22Rv1-AR-EK and CWR22Rv1 cells grown in steroid-depleted media were subject to control (siScr), total AR (siARex1) or AR-V only (siAR-V) knockdown for 96 hours before 10 x magnification bright field images were taken.

Supplementary Figure S9. CWR22Rv1-AR-EK RNA sequencing demonstrates down-regulation of DDR-associated genes in response to AR-V depletion. Expression of the 41 DDR-associated genes
identified to be consistently and significantly down-regulated (* p<0.01) in response to AR-V knockdown.

**Supplementary Figure S10. Validation of DDR-associated gene regulation by AR-Vs.** a. CWR22Rv1-AR-EK cells depleted of AR-Vs (siARex1) for 48 hours were subject to quantitative RT-PCR to assess expression of several DDR-associated genes. Data represents the average of three independent experiments +/- SD (* p < 0.05 as determined using a two-tailed student T-test).

**Supplementary Figure S11. Defining individual DNA repair pathways of the AR-V regulated DDR-associated genes.** The 41 DDR-associated genes demonstrated to be regulated by AR-Vs in the CWR22Rv1-AR-EK cell line were sub-compartmentalised into their associated DNA repair pathway using FunRich.

**Supplementary Figure S12. Expression AR-V-regulated DDR-associated genes is elevated in CRPC.** Expression of several AR-V-regulated DDR-associated genes from the CWR22Rv1-AR-EK cell line was assessed in benign hyperplasia (BPH), localised PC and CRPC samples *in silico* using the Grasso et al., (2012) data set (ns is non-significant; *, **, ***, **** represent p< 0.05, 0.01, 0.001 and 0.0001, respectively).

**Supplementary Figure S13. The presence of AR-V7 in patient samples correlates with elevated expression of several DDR-associated genes.** a. Expression of the 41 AR-V-regulated DDR-associated genes identified in CWR22Rv1-AR-EK cells were assessed in the TCGA dataset using cBioPortal with the presence of AR-V7 applied as an additional clinical parameter to enable quantification of expression in samples positive and negative for the receptor splice variant. b. Of the 41 genes, 9
demonstrated significantly elevated expression in PC samples that co-expressed AR-V7 (n=84) as calculated using a Bioconductor TCGAbiolinks package (version 2.9.0).

**Supplementary Figure S14. Overlapping DDR-associated genes between AR-Vs and FL-AR.** Venn diagram demonstrating overlapping DDR-associated gene signatures driven by AR-Vs in CWR22Rv1 cells (Jones et al., 2015) and FL-AR in LNCaP cells (Polkinghorn et al., 2012).

**Supplementary Figure S15. Examining ATM activation status in CWR22Rv1-AR-EK cells depleted of AR-Vs.** CWR22Rv1-AR-EK cells were transfected with control (siScr) or AR-targeting (siARex1) siRNAs for 24 hours before 2 Gy irradiation treatment and incubation for an additional 24 hours before immunoblotting using total ATM, phospho-ATM, AR and β-actin antibodies.

**Supplementary Figure S16. AR-V depletion in CWR22Rv1 cells reduces expression of ‘BRCAness’ genes.** CWR22Rv1 cells grown in steroid-depleted medium were transfected for 24 hours with control (siScr) or AR-targeting siRNAs (siARex1) before 24 hour treatment with and without 10 µM enzalutamide (Enz) were subjected to quantitative RT-PCR to assess ‘BRCAness’ gene expression. Data is presented as a heatmap (upper panel) and in graphical form; both represent the mean +/- SD of three independent experiments (* p<0.05 as determined using a two-tailed student T-test).

**Supplementary Figure S17. AR-V depletion in CWR22Rv1-AR-EK cells reduces expression of ‘BRCAness’ genes.** CWR22Rv1-AR-EK cells grown in steroid-depleted medium were transfected with control (siScr) or AR-V-targeting siRNAs (siAR-V) for 48 hours and were subjected to quantitative RT-PCR to assess gene expression. Data represent the mean +/- SD of three independent experiments.
(*p<0.05 as determined using a two-tailed student T-test). Parallel samples were subject to immunoblotting using AR and α-tubulin antibodies.

**Supplementary Figure S18. PARP inhibition using talazoparib reduces AR-V activity.** CWR22Rv1-AR-EK cells were treated with 1 μM talazoparib (Talaz) for 24 hours before quantitative RT-PCR to assess AR-V target gene expression. Data represents three independent experiments +/- SD (**p<0.001 as determined using a two-tailed student T-test). Parallel samples were subject to immunoblotting using AR and α-tubulin antibodies.

**Supplementary Figure S19. PARP inhibition using rucaparib reduces AR-V activity.** CWR22Rv1-AR-EK and CWR22Rv1 cells were treated with 0.5 and 1 μM olaparib (Olap) for 24 hours before quantitative RT-PCR to assess AR-V target gene expression. Data represents three independent experiments +/- SD (**p<0.001 as determined using a two-tailed student T-test).

**Supplementary Figure S20. PARP inhibition reduces AR enrichment at target genes.** CWR22Rv1-AR-EK (a.) or CWR22Rv1 cells (b.) treated for 4 (and 8) hours with 1 μM talazoparib (Talaz) were subject to ChIP using either anti-AR or control (IgG) antibodies to assess AR enrichment at AR target gene promoters PSA and KLK2. Data represents two independent experiments +/- SD (*, ** p<0.05, 0.01, respectively, as determined using a two-tailed student T-test). Immunoblotting of resultant CWR22Rv1 cell lysates using AR and α-tubulin antibodies is shown in the right panel.

**Supplementary Figure S21. PARP inhibition reduces AR enrichment at target genes.** CWR22Rv1-AR-EK cells were treated with and without 1 μM talazoparib (Talaz) for 4 hours before ChIP using PARP1/2 and control (IgG) antibodies to assess protein enrichment at AR target gene promoters PSA and KLK2,
and the **TMPRSS2** enhancer. Data represents two independent experiments +/- SD (** p<0.01 as determined using a two-tailed student T-test).

Supplementary Figure S22. Effect of ectopic AR-V7 and PARP1/2 inhibition on DDR-associated gene expression in LNCaP cells. LNCaP cells transiently transduced with control or AR-V7-expressing lentivirus for 24 hours and then treated with 1 μM talazoparib (Talaz) for an additional 24 hours were subject to quantitative RT-PCR to assess expression of DDR-associated genes. Data represents three independent experiments +/- SD (** p<0.01 as determined using a two-tailed student T-test). Genes were segregated into those up-regulated by ectopic AR-V expression and sensitive to PARP inhibitor (top left panel); those enhanced by AR-V7, but insensitive to PARP blockade (top right panel); and those AR-V independent (bottom panel).
### Supplementary Table S1 (up-regulated genes in response to AR-V depletion)

| Gene  | Gene  | Gene  | Gene  | Gene  | Gene  |
|-------|-------|-------|-------|-------|-------|
| AATK  | AKR1A1| ABCD1 | ACE   | ACTL10| ABHD14B|
| ADAT3 | ATP7A | AMOT1 | ANKR16| ALX4  | ABTB1  |
| AGT2A | BDH2  | AMPD2 | ASTN2 | ANKR52| ADCK5  |
| BAH1  | C4orf48| ARAP2 | BAIAP2L| ANXA9 | AMIGO2 |
| BID   | CA11  | ARNT2 | BPNT1 | APPL2 | ANG    |
| C1orf103| CALCOCO1| BICD2 | C1orf115| AR5  | ASS1  |
| C1orf24| CAMK2N1| BTBD9 | CDH7  | ATXN7L1| BOK    |
| C6orf32| CBX6  | C2orf118| CFD  | BSRY  | CACFD1 |
| C7orf43| CLN8  | C4orf32| CYP1A1| CASP7 | CDKN1A |
| CARD4 | CLTB  | CAMK1 | DBN1  | CD1PT | CHRM3  |
| CARN51| CRB3  | CAMLG | DDN   | CDK8  | CLSTN3 |
| CBL  | CREBL2| CTCL  | DNAJC18| CHFR  | CYB561D1|
| CHRN1E| DNAL  | CTXN1 | DSC2  | COL5A2| DDHD1  |
| COBL  | DUSP2 | CUEDC1| EFN1A | DNASE1| DUSP16 |
| DBC1 | EEF1A2| DACH1 | EML6  | DOPEY2| EFNAN3 |
| DCAF1L2| EID1  | DEGS2 | EPRB3 | ELOM3 | FAM102A|
| DEAF1 | EPAS1 | DHR53 | ERGIC1| EPHB3 | GLYCTK |
| DEND5A| EPN3  | DOCK4 | ERO1L  | FM109A| GMIP    |
| DGL5  | FGF3  | ENTPD6 | FAM63A| FAM111A| HIST3H2A|
| ETV4 | FKRP  | ERBB4 | FAM86HP| FGD3  | HOXC10 |
| EVPL  | FOSL2 | FAM173A| FNIP2  | FXYD3 | HSPG2  |
| FAM162A| G3BP2 | FERMT3 | GADD45G| GRAMD1A| IKZF2  |
| FAM84A| HEXIM1| GPR160 | GSTT1  | HIST1H2AC| IL17RE |
| FKB8P8| IFI35 | GYS1  | HES7   | HOXC13  | ITGAE  |
| FZD4  | IFT27 | KLC3  | ICA1   | IRAK2  | IZUMO4 |
| GGT1  | KDM6B | LOC646826| ID1  | ITGAE5 | JOSD2  |
| GREE5B1| KIAA0513| MIR600HG  | JMD7 | KHYNY  | JUN    |
| HIST2HBE| KLF4  | MTHFR  | JUP    | LOC284578| KDM2A  |
| IGFBP3| KLHL28| MVP   | KIF9   | LIX    | KIAA0922|
| LAMB2 | LDHD  | MYOIF | LAT2   | MAPK15 | LLGL2  |
| LITAF | LRFN4 | NCOA3  | LOC283335| MEX3D | LOC729737|
| LPPR2 | LYPLA2| NGEF | LRSAM1  | MICAL1  | MAP1LC3A|
| MROH6 | LZTR1 | NR1P1 | MAN2A2 | MRRA8 | MPP24  |
| MXD4  | MANSC1| OSGIN1 | MAN2B1 | MYH14  | NACC2  |
| MZF1  | MAPKAPK3| PAN3  | MFS6D  | NKAIN1 | NFKB1Z |
| NADK  | MARK1 | PANX2 | NCK2   | NOXA1  | PLEKHA2 |
| NUDT22| MC1R  | PCDHA10| PCBP4  | NPDC1  | POMGNT1|
| PLC3D | MFS6D10| PERP | PSMB80 | NUDT15 | PPRG2  |
| PLXNA1| MP2L3 | PKP2  | PTRPK  | PCHD19| RAB30  |
| PODXL2| NAP1L3| PLEKHA6| RBPMS | PCDH9  | RUNDC3B|
| POLD4 | NAPRT1| PNPLA6| REEP2  | PCNXL3 | SDC4   |
| PRAC  | NDUFB4| PPP2R2A| RHOC  | PDE4B  | SEMA3C |
|       |       |  |       |       | PTGS2  |
|       |       |  |       |       | PRPH   |
PRRT3 PDF PRMT6 S100P PDLIM2 SGSM3 RAB17 PTPRCAP
PVT1 PHF12 PROM2 SERTAD1 PSD3 SH3GL2B RENBP PTRH1
QDPR PITPNM1 RAB24 SGSM1 PTGFR SIX2 RHNPI1 RALYL
RAB11FIP4 PPP2R5A RNF11 SH2B1 PVRL4 SLC26A11 SATB1 RDH5
RASEF PTPN21 SAMD10 SH3BGR2 RAB3B SLC7A8 SH3RF1 RELB
RFX3 RILP SAT1 SITD2 RBMS2 SLT1 SLC30A10 RNF208
ROBO1 SLC29A4 SEMA3F SLC12A6 RIT1 SOX9 SLC40A1 SGK2
RSPH1 SLC41A2 SIGIR SLC25A29 RNPEPL1 SPHK2 SPRYD3 SI
S100A11 SLC6A6 SLC52A3 SLC39A13 RWDD2A STK40 SSSH3 SLC17A5
SCAND1 SOWAHB SLC5A6 SP110 Selm STX12 SYT7 SLC43A2
SHC4 SYTL1 STARD10 STOM SNX32 SYNGR2 TLL1 SLITRK5
SLC48A1 TEP1 STBD1 STXBP5 SPRY1 TBX2 TM7SF2 SSTR1
TMEM135 TET3 SYNJ2BP TJ2 JP TBHS1 TJP3 TNFRSF12A STON1
TNFRSF21 TINAGL1 TMEM184B TM6C TMEM79 TPM4 TNFSF9 TMC4
TRPV3 TMEM8A TPDS2L1 TMEM238 TNK2 TSTD1 TRPM4 TMEM125
TYRO3 WWP2 UNC13B TRADD TP53INP2 VASN TRPS1 TNFSF15
WNT9A XK8 WDR45 TTBB2 TULP4 ZNF385B TCC39A TRIB1
ZBTB7A ZDHHC18 ZG16B UNC45A ZFP36 ZNF524 UUK1 ZCCHC3
ZFP36L2 ZNF341 ZSCAN16 VGLL4 ZFYVE21 ZNF827 ZFP36L1 ZSWIM4

AATK AKR1A1 ABCD1 ACE ACTL10 ABHD14B ACBD4 ABCA1 APCDD1 AHR
ADAT3 ATP7A AMOT1 ANKRD16 ALX4 ABT1 ACPP ADP ATM1 ARHGAP32
AGT2A BH2D AMPD2 ASTN2 ANKRDS2 ADK5 ADAM15 ALOX15 BAMBI BASP1
BAHD1 C4orf48 ARAP2 BAIA2L2 ANX9 AMIG02 ADAMTS1 AMER3 BCRP2 C11orf95
BID CA11 ARNTL2 BPNT1 APPL2 ANG AES ANXA1 C19orf21 C8orf4
C17orf103 CALCOCO1 BID2 C1orf115 ARS1 ASS1 ARHGEF3 AQP3 C2orf15 C9orf152
C19orf24 CAMK2N1 BTBD9 CDH7 ATXN7L1 BOK ATP2B1 B3GNT3 CDC159 CACNA1D
C6orf132 CBX6 C2orf118 CF2 BSRY CACFD1 ATP8A1 CDC42EP3 CDH18 CDC64B
C7orf43 CLN8 C4orf32 CYP1A1 CASP7 CDKN1A C15orf57 CNT3 CLN1 CDC120 CDC68B
CARD14 CLT8 CAMK1 DBN1 CDIP CHRM3 CCDC120 CREG3L1 CLN4 CDH12
CARN5I CRB3 CAMLG DDN CDK8 CLSTN3 CPEB3 DSCAM-AS1 CYP4F35P CHST2
CBLC CREBL2 CTC1 DNAJC18 CHFR CYB561D1 CYSTM1 ELF3 DCUN1D COL4A1
CHRNE DNLN1 CTX1 DSC2 COL5A2 DDHD1 DSP1 ENPP4 DSP4 CTTNBP2
COBL DUSP2 CUEDC1 EFNA1 DNASE1 DUSP16 Dyrk1B FBLIM1 FAM110C CYP1A2
DBC1 EEFA2 DACH1 EML6 DOPEY2 EFNB2 EFNA3 FJI38109 FJU22184 DDIT4L
DCAF1L2 EID1 DEGS2 ERBB3 ELMO3 FAM102A EGR1 GAB2 FLJ23867 DGKA
DEAF1 EPSA1 DHR53 ERGIC1 EPHB3 GLYCTK FAM195B GATA2 FOXO4 ELF5
DENNDA4 EPN3 DOCK4 ERO1L FAM109A GMIP FAM214B GPR3 FOXP4 ENPPS
DING5 FGFR3 ENTPD6 FAM63A FAM111A HIST3H2A FLJ20021 HABP4 GDPD1 EVX1
ETV4 FKRP ERB4 FAM86HP FGDO3 HOXC10 GABARAP1 HGD GPR35 FOS
EVPL FOSL2 FAM173A FNIP2 FXD3 HSPG2 HIST1H3H HID1 GUSPB1 GALNT3
FAM162A G3BP2 FERM3 GADD45G GRAMOD1A IKZF2 HOXA13 HIST1H2AG HGF GSN
FAM84A HEXIM1 GPR160 GST11 HIST1H2AC IL17RE IL17RC HIST1H2BK HIST1H1C HMGCS2
FKBP8 IFI35 GYS1 HES7 HOXC13 ITGAX IMMP2L KCND2 HIST1H3E HOXC12
## Supplementary Table S2 (down-regulated genes in response to AR-V depletion)

| AAED1 | ACADL | ACBD7 | ADORA1 | AAK1 | AADAT | AFAP1L1 | ABHD15 | ALDH1L2 | ARL1 |
|-------|-------|-------|--------|------|-------|----------|--------|---------|------|
| GGT1  | KDM6B | LOC646862 | ID1 | ITGAV | JOSD2 | LAD1 | KIAA1522 | IL36RN | KIAA1199 |
| GREB1L | KIAA0513 | MIR600HG | JMJD7 | KHNYN | JUN | LOC113230 | KRT8 | INPP5A | KIAA1324 |
| HIST2H2BE | KLF4 | MTHFR | JUP | LOC284578 | KDM2A | LOC338758 | LANC3 | JUNB | LCOR |
| IGFBP3 | KHLH28 | MVP | KIF9 | LNX | KIAA0922 | MAFK | LOC100862671 | KCNJ11 | LNX1 |
| LAMB2 | LDHD | MYOF | LAT52 | MAPK15 | LLGL2 | MAP15 | LOC388692 | KCNJ3 | LOC286367 |
| LITAF | LRNF4 | NCOA3 | LOC283335 | MEX3D | LOC729737 | MAPRE2 | LRPL0 | KIF13B | MAFF |
| LPPR2 | LYPLA2 | NGEF | LRSAM1 | MICAL1 | MAP1LC3A | MNT | MAPK13 | KLHL1 | MAPK4 |
| MROH6 | LZR1 | NRP1 | MAN2A2 | MXRA8 | MMP24 | MTS51L | MST4 | LHX9 | MFS4 |
| MXD4 | MAN5C1 | OSGIN1 | MAN2B1 | MYH14 | NACC2 | MYRIP | MUC1 | LMA1 | NCMAP |
| MZF1 | MAPKAPK3 | PAN3 | MFS6D | NKA1N1 | NFKB1Z | NCAM2 | MYO7A | LIPH | NOTCH3 |
| NADK | MARK1 | PANX2 | NCK2 | NOXA1 | PLEKHA2 | NR1H2 | Pced1B | LOC10012770 | NOV |
| NUDT22 | MC1R | PCDAH10 | PCBP4 | NPD1C | POMGNT1 | PCDAH4 | PEG10 | LRRK56 | OGRF |
| PLCD3 | MFSD10 | PERP | PSM1B | NUDT14 | PRRG2 | PEX11A | PEL1 | MAL2 | OSBPL5 |
| PLXNA1 | MPZL3 | PKP2 | PTRPK | PCDH19 | RA830 | PLEKHA7 | PHLD03 | MESDC2 | PAN3-A51 |
| PODOXL2 | NAP1L3 | PLEKHA6 | RBPMS | PCDH9 | RuncDC3B | PLEKHB1 | PIK3AP1 | MESP1 | PART1 |
| POLD4 | NAPRT1 | PNPLA6 | REEP2 | PCNJL3 | SDC4 | PLXNA2 | PLXNB3 | NKD1 | PLAG2A4 |
| PRAC | NDUFB4 | PPP2R2A | RHOC | PDE4B | SDA3C | PTGS2 | PRPH | PBIP1 | PLAG24F |
| PRRT3 | PDF | PRMT6 | S100P | PLD1M2 | SGS3M | RAB17 | PTRPCAP | PLA2G16 | PPFA2 |
| PVT1 | PHF12 | PROM2 | SERTAD1 | PS3D | SH3GL2B | RENBP | PTRH1 | PNCK | RALGAPA2 |
| QO9DR | PITPNM1 | RAB24 | SGSM1 | PTGFR | SIX2 | RHNPL1 | RALY | PNPLA7 | REG4 |
| RAB11FIP4 | PPP2R5A | RNF11 | SH2B1 | PVR14 | SLC26A11 | SATB1 | RDS5 | PPP1R38 | SCUBE2 |
| RASEF | PTPN21 | SAMD10 | SH3BGRL2 | RAB3B | SLC7A8 | SH3RF1 | RELB | PTHLH | SLC5A8 |
| RXF3 | RELP | SAT1 | SIT2 | RBMS2 | SLT1 | SLC30A10 | RNF208 | QSOX1 | SPEF1 |
| ROBO1 | SLC29A4 | SDA3F | SLC12A6 | RIT1 | SOX9 | SLC40A1 | SGK2 | RAB25 | STAP2 |
| RSPH1 | SLC41A2 | SIGIRR | SLC25A29 | RNPEPL1 | Sphyk2 | SPRYD3 | SI | RBM11 | SULT2B1 |
| S100A11 | SLC6A6 | SLC52A3 | SLC39A13 | RWD2DA | STK40 | SSH3 | SLC17A5 | RNF223 | SYT4 |
| SCAND1 | SOWAH8 | SLC5A6 | SP110 | SELM | STX12 | SY77 | SLC43A2 | SLC50A1 | SYT2 |
| SHC4 | SYT1 | STARD10 | STOM | SNX32 | SYNGR2 | TLL1 | SLTRK5 | SMPDL3B | TLE1 |
| SLC48A1 | TEPI | STBD1 | STXBPS | SPRY1 | TBX2 | TM7SF2 | SSRT1 | TMEM2 | TMEM45B |
| TMEM135 | TET3 | SYNJ2BP | TIP2 | THBS1 | TJIP | TNFRSF12A | STON1 | TNFRSF11B | TNFRSF19 |
| TNFRSF21 | TINAGL1 | TMEM184B | TMC6 | TMEM79 | TPM4 | TNFRSF9 | TMC4 | TPS3INP1 | TSPAN1 |
| TRPV3 | TMEM8A | TPSD2L1 | TEMD238 | TNK2 | TSTD1 | TRPM4 | TMEM125 | TSPAN12 | ZDHHC16 |
| TYRO3 | WW2P | UNC13B | TRADD | TPS3INP2 | VASN | TRPS1 | TNFRSF15 | TSPAN15 | ZNF385A |
| WNT9A | XKR8 | WDR45 | TTBK2 | TULP4 | ZNF385B | TTCA9 | TRIB1 | TUF1 | |
| ZBTB7A | ZDHHC18 | ZG16B | UNC45A | ZFP36 | ZNF524 |ULK1 | ZCCHC3 | ZCCHC3 | |
| ZFP36L2 | ZNF341 | ZSCAN16 | VGLL4 | ZFYVE21 | ZNF827 | ZFP36L1 | ZSWIM4 | ZCCHC24 | |
| Biological process                  | No. of genes | % of genes | Fold enrichment | P-value (Hypergeometric test) | Bonferroni method (corrected p-value) |
|------------------------------------|--------------|------------|----------------|-----------------------------|--------------------------------------|
| cell division                      | 63           | 9.81       | 4.96           | 1.77393E-26                | 2.10849E-22                         |
| DNA-dependent DNA replication      | 7            | 1.09       | 8.46           | 1.12367E-05                | 0.133559243                         |
DNA-dependent DNA replication initiation

| Event                                      | Value1 | Value2 | Value3 | Value4 | Value5 |
|--------------------------------------------|--------|--------|--------|--------|--------|
| DNA-dependent DNA replication initiation   | 17     | 2.65   | 13.12  | 1.0575E-15 | 1.25696E-11 |
| DNA replication                            | 34     | 5.30   | 6.95   | 1.43037E-19 | 1.70013E-15 |
| mitotic cell cycle                         | 20     | 3.12   | 4.56   | 1.35163E-08 | 0.000160655 |
| G1/S transition of mitotic cell cycle      | 21     | 3.27   | 5.61   | 1.0804E-10 | 1.28416E-06 |
| regulation of transcription involved in G1/S phase of mitotic cell cycle | 11     | 1.71   | 13.29  | 1.10301E-10 | 1.31104E-06 |
| G2/M transition of mitotic cell cycle      | 19     | 2.96   | 4.06   | 2.05028E-07 | 0.002436959 |

Cell Division

KOHL42; NCAPG2; CDC25C; KIF11; RB1; CCNE2; MIS18A; CCSAP; CEP63; KNSTRN; NTC1; MAD2L1; CETN3; ITGB3BP; CDC48; REEP4; CDC7; MASTL; LRRCC1; MIS18BP1; REEP3; CENPJ; ERCC6L; NU62; CDC6; KIF20B; HLLS; GNAI2; SMAC; KIF14; EPB41; DYNLT1; CDK1; NCAPG; BORA; SKE3; NUP43; CCNB2; SMC2; SMCA1A; CCNA2; NDC80; HAUS3; SPAG5; KIF18B; BUB1B; CCNB1; SPDL1; UBE2C; FBXO5; HAUS6; TCC28; CDC20; LG1; ZWINT; BOD1; SPC25; CENP; ZWILCH; NEDD1; CCNE1; PDS5B; CENPF;

DNA-dependent DNA replication

POLE2; POLQ; RFC3; WDHD1; RFC5; RFC4; POLA1;

DNA-dependent DNA replication initiation

CCNE2; POLE2; MCM8; MCM10; CDC7; PRIM1; MCM3; CDC6; POLA2; GINS4; POLA1; MCM6; ORC3; ORC1; MCM4; PRIM2; CCNE1;

DNA replication

RRM1; CDC25C; CLSPN; BARD1; RRM2; POLE2; MCM8; TICRR; DNA2; BRIP1; MCM10; BRCA1; CDC7; DTD1; MCM3; BLM; EXO1; RAD1; CDC6; RFC3; RFC5; RMI1; CDK1; RFC4; RHNO1; DONSON; POLA2; POLA1; MCM6; ORC3; FEN1; RMI2; ORC1; MCM4;

Mitotic cell cycle
RRM1; KIF11; CIT; MYBL1; PBK; DNMT3A; KIF15; MASTL; CDC6; WDHD1; SKA3; NDC80; XRCC2; CLIP1; KIF18B; BUB1B; PBRM1; CENPE; TUBE1; CENPF;

**G1/S transition of mitotic cell cycle**

RB1; CCNE2; POLE2; MCM8; MCM10; CDC7; PRIM1; MCM3; CDC6; RPS6KB1; CUL4B; POLA2; POLA1; MCM6; ORC3; ORC1; MCM4; EIF4EBP1; PRIM2; CCNE1; CDKN3;

**Regulation of transcription involved in G1/S phase of mitotic cell cycle**

PCNA; RRM2; CDC6; KLF11; TYMS; POLA1; FBXO5; ORC1; E2F1; DHFR; CCNE1;

**G2/M transition of mitotic cell cycle**

CDC25C; CIT; FBXW11; CEP63; MASTL; CENPJ; CEP152; CDK1; BORA; CCNB2; CCNA2; HAUS3; MELK; CEP78; CCNB1; HAUS6; NES; NEDD1; PLK4;

**Supplementary Table S4**

| Biological process                                           | No. of genes | % of genes | Fold enrichment | P-value (Hypergeometric test) | Bonferroni method (corrected p-value) |
|--------------------------------------------------------------|--------------|------------|----------------|------------------------------|--------------------------------------|
| DNA synthesis involved in DNA repair                         | 15           | 2.34       | 11.91          | 3.13E-13                     | 3.73E-09                             |
| DNA repair                                                   | 32           | 4.98       | 4.23           | 4.63E-12                     | 5.5E-08                              |
| DNA ligation involved in DNA repair                          | 3            | 0.47       | 8.35           | 0.004611                     | 1                                    |
| DNA damage checkpoint                                        | 9            | 1.40       | 8.34           | 6.97E-07                     | 0.008287                             |
| DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest | 9            | 1.40       | 4.04           | 0.000354                     | 1                                    |
| double-strand break repair via homologous recombination      | 17           | 2.65       | 5.91           | 2.85E-09                     | 3.38E-05                             |

**DNA synthesis involved in DNA repair**

BARD1; DNA2; BRIP1; BRCA1; BLM; EXO1; BRCA2; RFC3; RAD51B; RAD51AP1; RMI1; RAD51; POLA1; XRCC2; RMI2;

**DNA repair**
DNA ligation involved in DNA repair

HMGB2; PARP2; LIG1;

DNA damage checkpoint

CLSPN; CEP63; CLOCK; BRIP1; RAD1; RHNO1; DONSON; USP28; E2F1;

DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest

PCNA; CNOT6; CDC25C; CENPJ; CDK1; SFN; CCNB1; E2F7; E2F1;

Double-strand break repair via homologous recombination

AUNIP; RAD54B; MCM8; RAD54L; MMS22L; POLQ; BRCA1; BLM; BRCA2; RAD51B; RAD51AP1; RAD51; GEN1; NUCKS1; XRCC2; FEN1; SMC6;

**Supplementary Table S5 (Primers used for qRT-PCR and ChIP)**

| Oligo Name   | Sequence (5'→3')                      |
|--------------|---------------------------------------|
| HPRT1 mRNA F | TTGCTTTCCCTGGTCAGGCA                  |
| HPRT1 mRNA R | AGCTTTGACCTTGACCATCT                  |
| UBE2C mRNA F | TGCCCTGTATGATGTCAGGA                  |
| UBE2C mRNA R | GGGACTATCAATGTTGGGTCT                 |
| PSA mRNA F   | GCAGCATTGAACCAGAGGAG                  |
| PSA mRNA R   | AGAACTGAGGAGGCGTTGAG                  |
| CCNA2 mRNA F | GAAGACGAGACGGTGTTGCA                  |
| CCNA2 mRNA R | AGAGAAGACGGTGACATGCT                  |
| KLK2 F mRNA  | AGCATCGAACCAGAGGTGTTCT                |
| KLK2 R mRNA  | TGAGGACTCAACACCACTGAAGA               |
| ATAD2 mRNA F | TGCGACCACTGCATCAT                    |
| ATAD2 mRNA R | AGCTTCAGGAATCACCCTGGG                 |
| FKBP5 mRNA F | CCCCTATTGTTATCCGGAGTAC                |
| FKBP5 mRNA R | TTGGGAAGACAGACACACCTG                 |
| TMPRSS2 mRNA F | CTGCTGGATTTCCCGGTG                 |
| Gene         | mRNA Type | Primer Sequence |
|--------------|-----------|-----------------|
| TMPRSS2      | mRNA R    | TTCTGAGGTCTTCCCTTCTCCT |
| FL-AR        | mRNA F    | AACAGAAGTACCTGTGCCGGC |
| FL-AR        | mRNA R    | TTCAGATTACCAAGTTCTTCAG |
| AR exon 3    | mRNA F    | AACAGAAGTACCTGTGCCGGC |
| AR-V1        | mRNA R    | TGAAGCTCAAACACCTCAG |
| AR-V3        | mRNA F    | AGACGAAGCTTCTGGGTG |
| AR-V3        | mRNA R    | CATGCAGTATGGCTGGG |
| AR-V5        | mRNA R    | CAAAGAAATTTGGAAGAAG |
| AR-V7        | mRNA R    | TCAGGGTCTGGTCATTTTGA |
| AR-V9        | mRNA R    | GCAAAATGTCTCAAAAAGCAG |
| DMC1         | mRNA F    | AGGTGCAAATGTTATACCC |
| DMC1         | mRNA R    | TGAAGACACTCGGCTC |
| XRC2         | mRNA F    | TCACCTGGTCATGGTGATTT |
| XRC2         | mRNA R    | TTCCAGGCACCTTCTGATT |
| RMI2         | mRNA F    | GCCAGGGTAGTAGTAGGGCAAG |
| RMI2         | mRNA R    | CCTGAACCACCTCCCCACTACCAT |
| BRCA1        | mRNA F    | CGTAAAGACTGCTGACGGC |
| BRCA1        | mRNA R    | AGGGTAGCTTGAAGGCTG |
| RAD51AP1     | mRNA F    | CTTCTGGAGAGCCTAGTG |
| RAD51AP1     | mRNA R    | AGAGAAGTCTTCGCTTATAT |
| RAD54L       | mRNA F    | CTTTCTCCATCCTGAGT |
| RAD54L       | mRNA R    | GCTTCAAGGCTGTAACAGG |
| CHEK1        | mRNA F    | GTGCAGTAGCTCCAGG |
| CHEK1        | mRNA R    | GTTCTGGCTGAGAAGACTG |
| EXO1         | mRNA F    | TCGGATCTCAGGTGG |
| EXO1         | mRNA R    | AGCTGTCTGCACATTCTAGCC |
| NBN1         | mRNA F    | TCTGTGAGGCCAGAGAAGAAG |
| NBN1         | mRNA R    | CACCTCAGAAAAGAAGGCAAG |
| RAD54B       | mRNA F    | GGTGTTGCAAGCTCCTAGC |
| RAD54B       | mRNA R    | AGCATATCCATGACGCTTACATAC |
| RAD51C       | mRNA F    | GTGAACCCCTCCAGCTG |
| RAD51C       | mRNA R    | CCTGCTCAAGAAGTCTCAGTGC |
| ABCF2        | mRNA F    | GAGGTTCCTAGGAGCAGAAG |
| ABCF2        | mRNA R    | CTGTAGGGTCTTCTCCTG |
| CLSPN        | mRNA F    | AAGGGAGCAATTGAAGGG |
| CLSPN        | mRNA R    | TCTGCAGGGTCTGAGCT |
| PCNA          | mRNA F    | GCCATATTGGAGATGCTG |
| PCNA          | mRNA R    | TGAGTGTCACGTTGAAG |
| BRCA2        | mRNA F    | GCTTTAAAAAGCACTCCAGAG |
| BRCA2        | mRNA R    | GATTCTGTATCCGCTTGAAG |
| RAD21        | mRNA F    | TCCCCCAGAGGACCTCCAA |
| Oligo Name       | Sequence (5'→3')                      |
|------------------|--------------------------------------|
| RAD21 mRNA R     | AGCAAGAGCTCGCTGGAGACCA                |

### Supplementary Table S6 (siRNA sequences used in study)

| Oligo Name | Sequence (5'→3')                      |
|------------|--------------------------------------|
| siARex1    | CAAGGGAGGUUACACCCAAA                  |
| siARex4    | CCAUCUUUCUGAAUGUCUU                   |
| siARex7    | GGAACUCGAAUCGUAUCAUUU                 |
| siAR-V     | GUAGUUGUGAGUAUCUGA                    |
| siScr      | UUCUCCGAACGUGUCAGU                   |
CWR22Rv1

*sgRNA_1: 116 bp+221 bp
*sgRNA_2: 128 bp+209 bp

**TIDE analysis**

| % Sequences | deletion | insertion |
|-------------|----------|-----------|
| sgRNA_1     | 21.1%    | 8.9%      |
| sgRNA_2     | 21.2%    | 8.8%      |

**Total eff.** = 32.4%

**Total eff.** = 20.1%

**α-tubulin**

Supplementary Figure S1
Supplementary Figure S2

**Supplementary Figure S2**

| IB       | AR-N20 | AR-BD | AR ab74272 | AR-441 |
|----------|--------|-------|------------|--------|
| Epitope (aa): | NTD 33-485 | NA 299-315 |

**Notes:**

- **AR-FL**
- **AR-Vs**
- **α-tubulin**

**Exposure:**
- **Short exposure**
- **Long exposure**
c.

**CRISPR off-target analysis: CWR22Rv1-AR-EK**

- **COL1A2**
  - CWR22Rv1
  - AR-EK
  - PAM

- **KMT2B**
  - CWR22Rv1
  - AR-EK
  - PAM

- **SLC7A8**
  - CWR22Rv1
  - AR-EK
  - PAM

**Cell line validation**

| Markers     | CWR22Rv1 | CWR22Rv1-AR-EK |
|-------------|----------|----------------|
| Amelogenin  | X,Y      | X,Y            |
| vWA         | 15,21    | 15,21          |
| TPOX        | 8        | 8              |
| THO1        | 6,9.3    | 6,9.3          |
| D21S11      | 30       | 30             |
| D5S818      | 11,12    | 11,12          |
| D13S317     | 9,12     | 9,12           |
| D7S820      | 10,11    | 10,11          |
| D16S539     | 12       | 12             |
| CSF1PO      | 10,11    | 10,11          |
Supplementary Figure S4
Supplementary Figure S5
Supplementary Figure S6
**Supplementary Figure S7**

**a.**

CWR22Rv1-AR-EK

![Bar chart showing normalized mRNA expression for PSA, TMPRSS2, UBE2C, and AR-V7 with siScr and siARex1 treatments.](image)

**b.**

![Results of Western blot analysis showing the mean of normalized counts for AR-Vs and β-actin with p < 0.01.](image)
Supplementary Figure S9
Supplementary Figure S10
Supplementary Figure S11
a. b.TCGA

Supplementary Figure S13
Supplementary Figure S14
CWR22Rv1-AR-EK

Irradiation (2 Gy):
- +

IB: Total ATM
IB: Phospho-ATM
IB: AR (N)

↓AR-Vs

β-actin

Supplementary Figure S15
Supplementary Figure S16
CWR22Rv1-AR-EK

Supplementary Figure S17
CWR22Rv1-AR-EK

Supplementary Figure S18
**CWR22Rv1-AR-EK**

a.

![Bar charts showing normalised mRNA expression for PSA, KLK2, UBE2C, and CCNA2 in CWR22Rv1-AR-EK cells treated with DMSO, 0.5 µM Ruc, and 1 µM Ruc.](image)

b.

![Bar charts showing normalised mRNA expression for PSA, KLK2, UBE2C, and CCNA2 in CWR22Rv1 cells treated with DMSO, 0.5 µM Ruc, and 1 µM Ruc.](image)

Supplementary Figure S19
CWR22Rv1-AR-EK

AR ChIP: PSA Prom

AR ChIP: KLK2 Prom

Fold % Input

CWR22Rv1

AR ChIP: PSA Prom

AR ChIP: PSA Enh

AR ChIP: KLK2 Prom

AR ChIP: TMPRSS2 Enh

Fold % Input

Goodness of fit:

DMSO
4h - Talaz (1μM)
8h - Talaz (1μM)
IgG

Supplementary Figure S20
**Supplementary Figure S22**

**AR-V-driven PARPi sensitive**

- RAD21 mRNA
- CHEK1 mRNA

**AR-V-driven PARPi insensitive**

- XRCC2 mRNA
- DMC1 mRNA
- EXO1 mRNA

**AR-V independent**

- RAD51AP1 mRNA
- RAD54L mRNA
- RAD51C mRNA
- RMI1 mRNA
- ABCF2 mRNA
- RAD54B mRNA