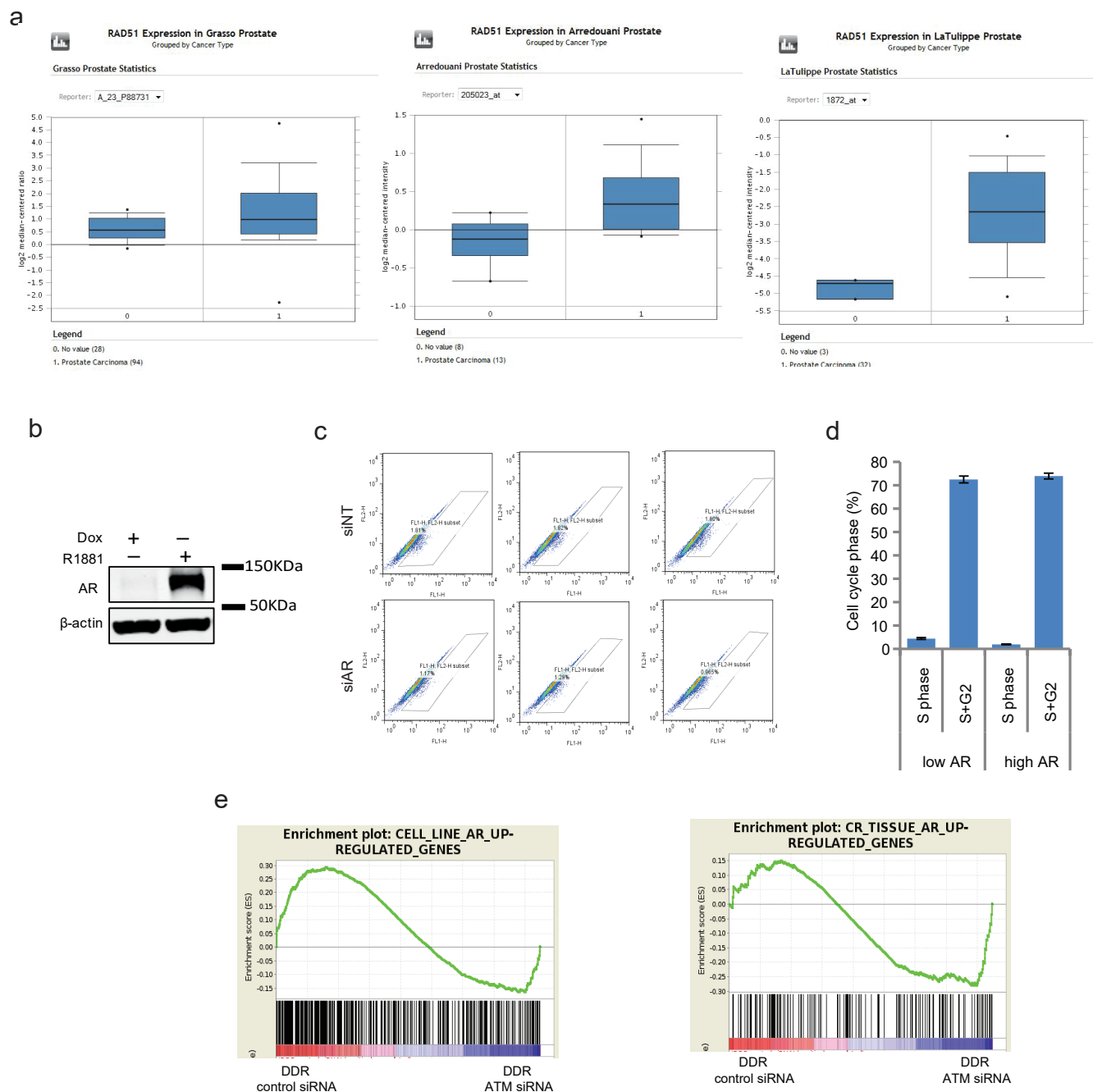


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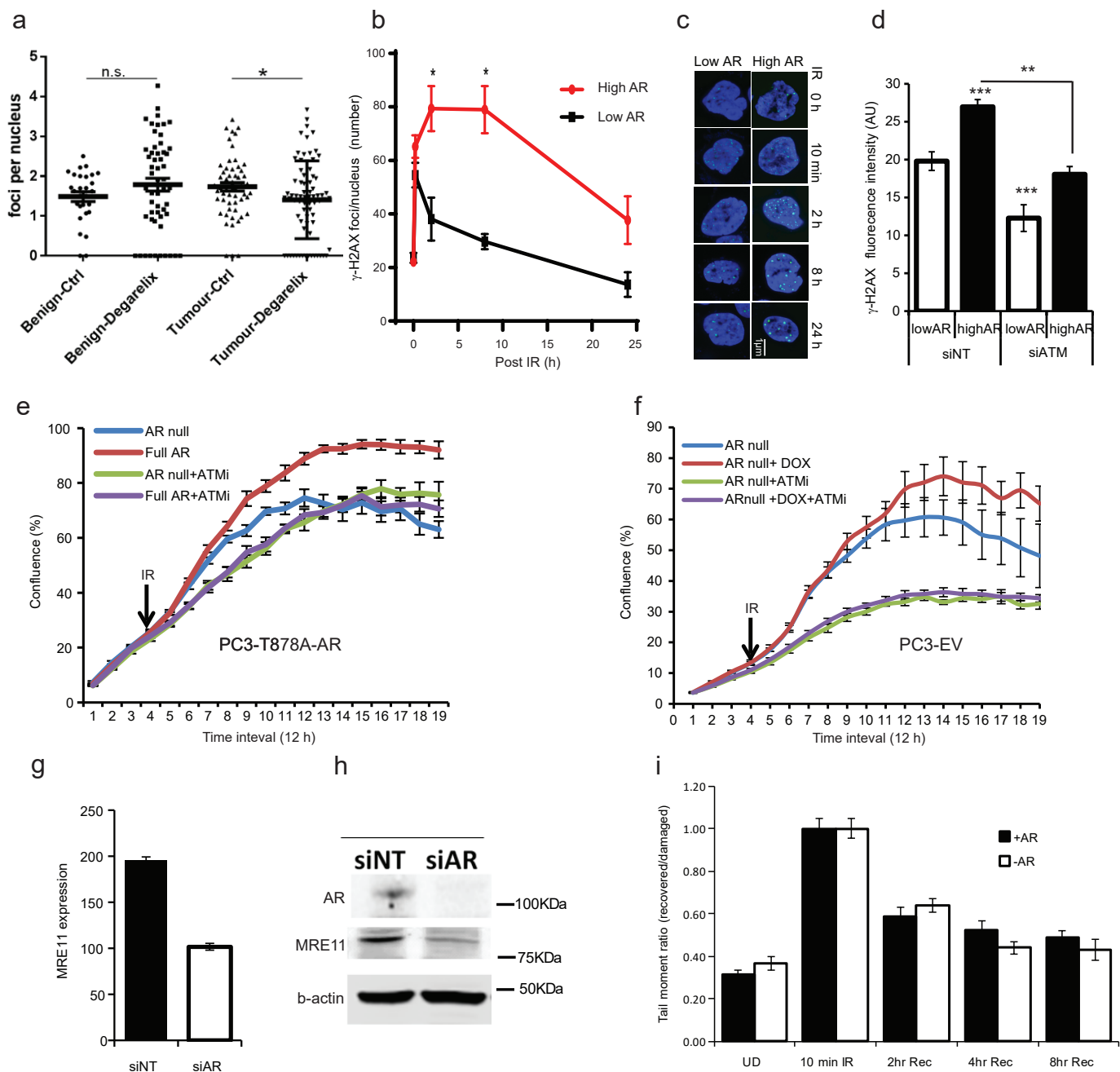
Description: Supplementary Figures, Supplementary Table and Supplementary References

File Name: Peer Review File

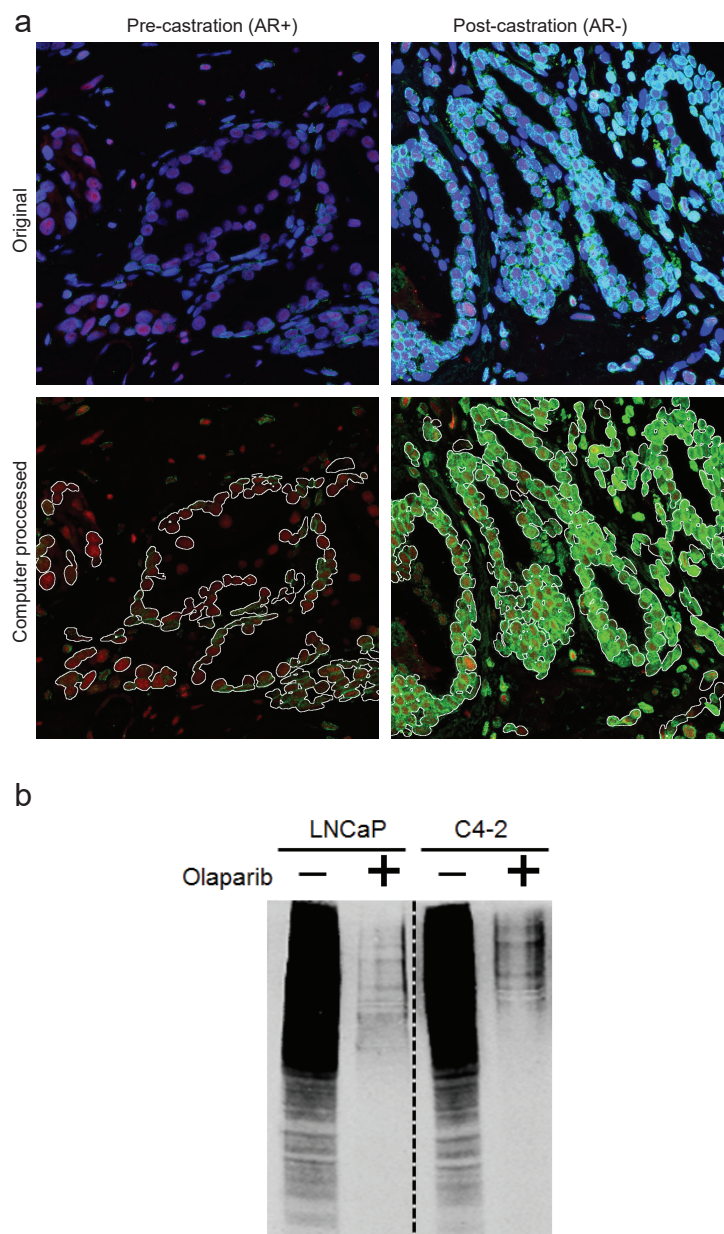
Description:



**Supplementary Figure 1. Upregulation of HR factors in prostate cancer and link with AR signalling.** (a) Distribution plot in Oncomine showing log2 expression value of RAD51 gene transcripts in three different PCa clinical gene expression data sets<sup>1-3</sup>. Plots show IQR, 95% CI and outlier points. (b) Western blot showing level of AR in “low AR” and “high AR” C4-2 cells treated with doxycycline (1 µg/ml) or androgen R1881 (1 nM) for 72 hr. (c) Flow cytometry plots for C4-2-DRGFP cells; boxes enclose cells which represent percentage of GFP positive cell population. (d) Flow cytometry data plotted as bar chart for cell cycle distribution (e) Gene set enrichment scores for DNA damage signatures for AR-regulated genes (AR occupied and down-regulated following castration in xenografts, GSE28126 and GSE21887).



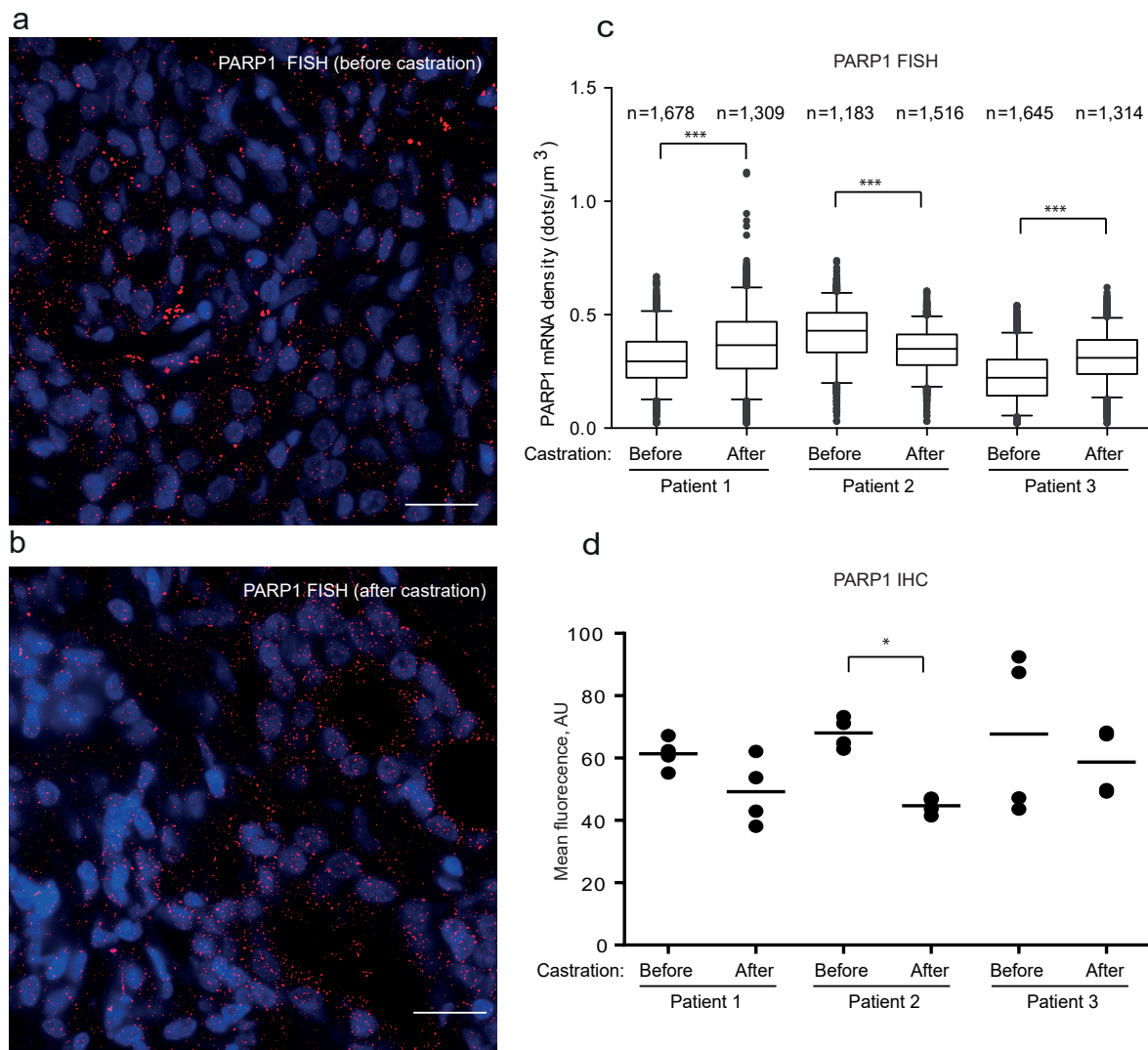
**Supplementary Figure 2. Effect of AR signaling on DDR pathway in prostate cancer.** (a) Scatter plot showing mean  $\gamma$ H2AX foci per nucleus from benign glands and PCa tissue from patients treated with degarelix (number of patients=15) or tumour matched untreated control (number of patients=19). Each dot represents the average number of foci per nucleus in individual glands. Statistical significance was calculated by unpaired two-tailed Student's t-test ( $p < 0.05$ ). (b) Foci analysis time course showing high content cytometry based quantification of the number of  $\gamma$ H2AX foci in "low AR" and "high AR" C4-2 cell nuclei in response to radiation (10 Gy) at indicated time points. Statistical significance determined using the Holm-Sidak method, with  $\alpha = 5.000\%$ . (c) Confocal microscopy images showing  $\gamma$ H2AX foci (green dots) in the nuclei of "high AR" and "low AR" C4-2 cells at the indicated time after exposure to radiation. P value by two-sided Student's t-test. (d) High content cytometry analysis histogram show mean  $\gamma$ H2AX levels per nucleus in "low AR" and "high AR" C4-2 cells transfected with siNT (non-targeting control) or siATM and measured 2 hr after exposure to radiation (10 Gy); data are presented as  $\pm$  SEM. (e) Live cell imaging confluence analysis (Incucyte) of a dox-inducible mutant AR (T878A) expressing PC3 cell line (full AR) and its null counterpart (AR null) treated with ATMi (10  $\mu$ M) for 2.5 days prior to and for 7 days following exposure to radiation (10 Gy as indicated by an arrow), standard error of mean is presented. (f) Live cell imaging confluence analysis (Incucyte) of AR null, PC3 empty vector cell line treated with doxycycline (1  $\mu$ g/ml) and/or ATMi (10  $\mu$ M) and exposed to radiation after 48 hr as indicated by arrow (10 Gy) for a total 156 hr; data represented as  $\pm$ SEM. (g) Histogram showing MRE11 expression in LNCaP cells transfected with control siRNA (siNT) or siAR; data represented as  $\pm$ SEM. (h) Western blot of C4-2 cells transfected with control siRNA (siNT) or siAR, showing MRE11 protein expression under the stated conditions, with  $\beta$ -actin as the internal control. (i) Neutral comet assays in "high AR" and "low AR" cells.



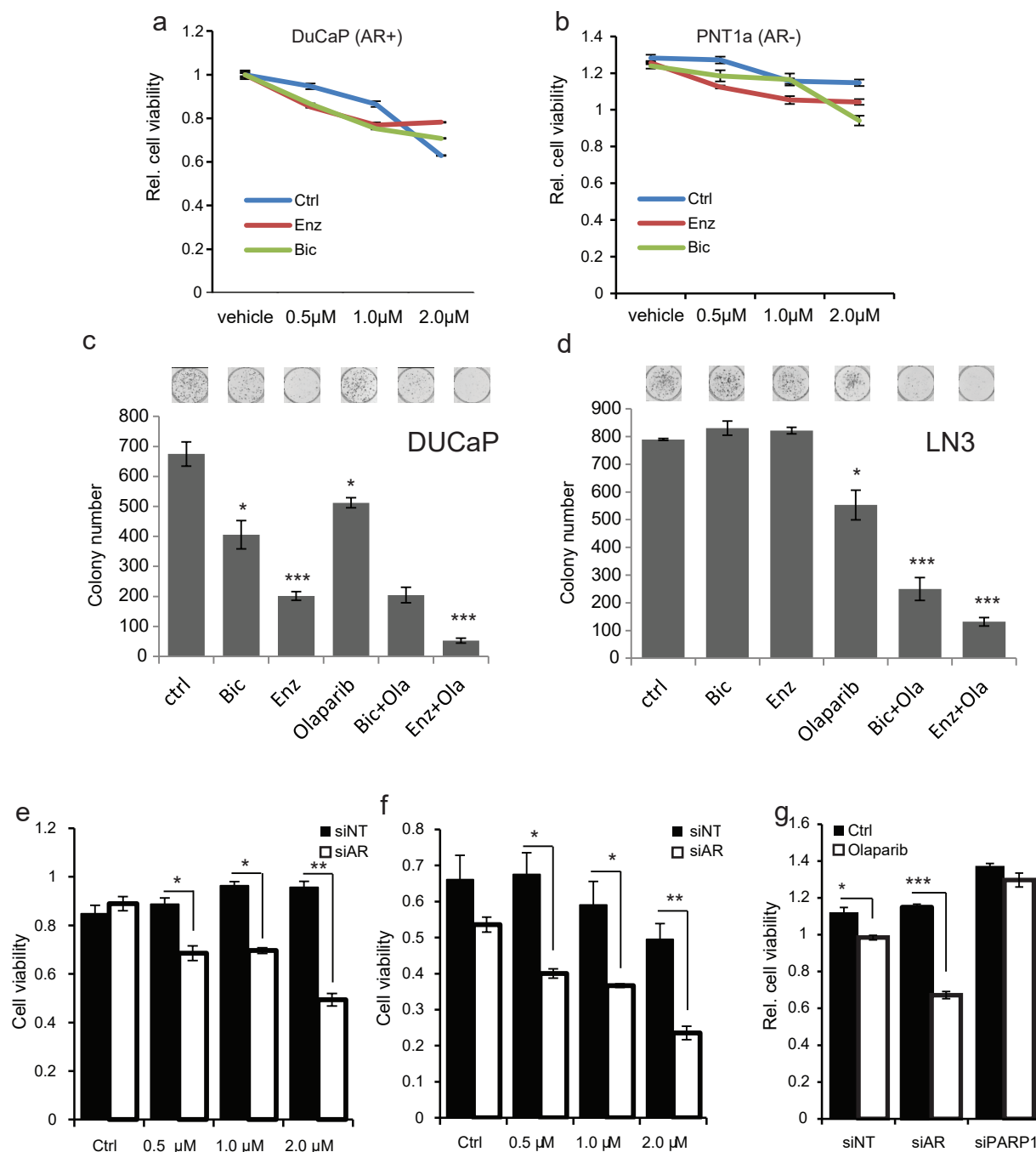
**Supplementary Figure 3. PARP expression in prostate cancer cell lines and tissue.**

**(a)** Immunofluorescence images showing original and computer processed images of the PCa tissue stained for PARP1 (red), PAR (green) and DNA (blue). White lines delineate the areas used for intensity measurement. **(b)** Western blot showing PARylated proteins from LNCaP and C4-2 cells treated with PARP-1/2 inhibitor Olaparib (1  $\mu$ M) for 24 hr.

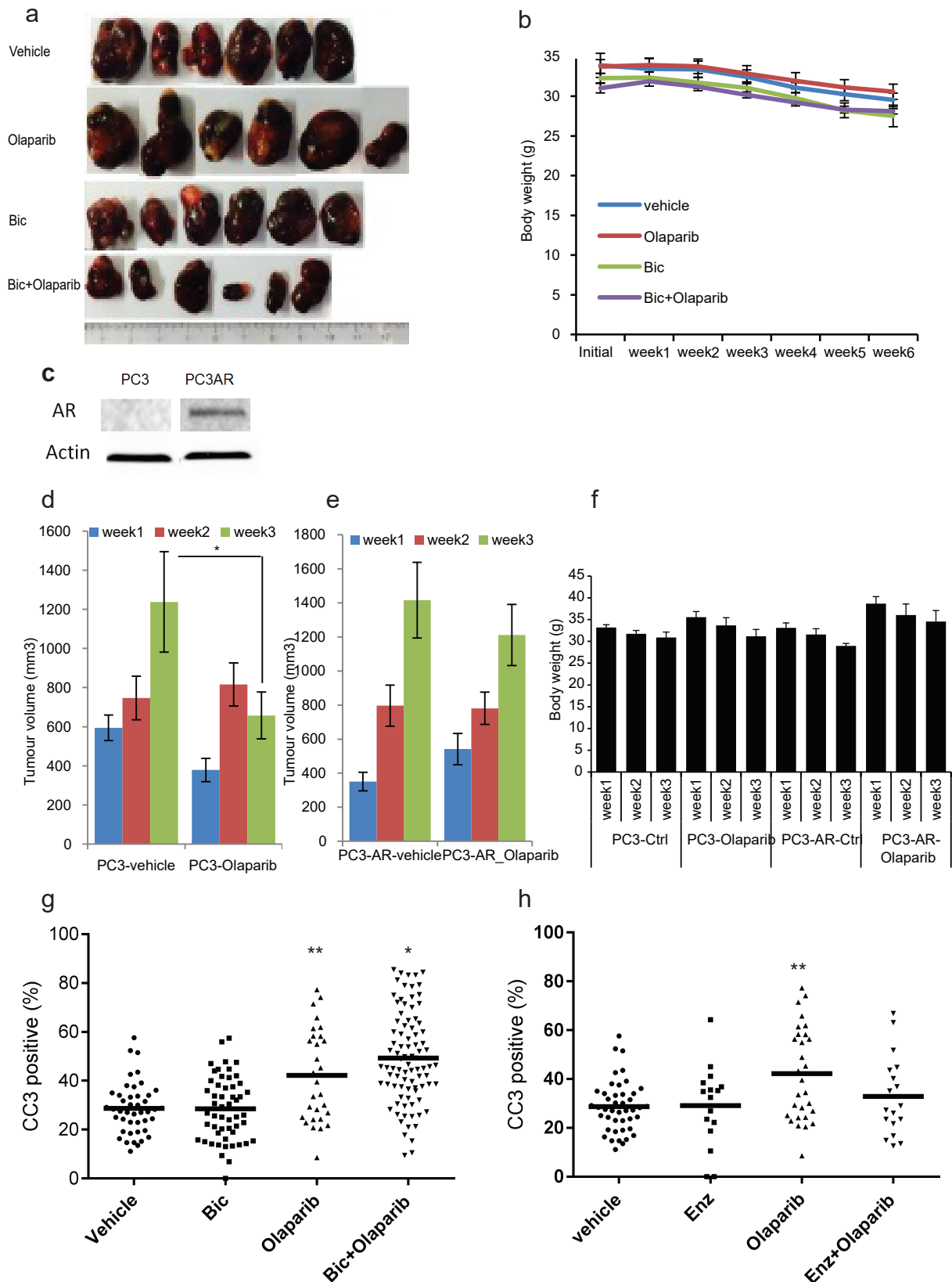




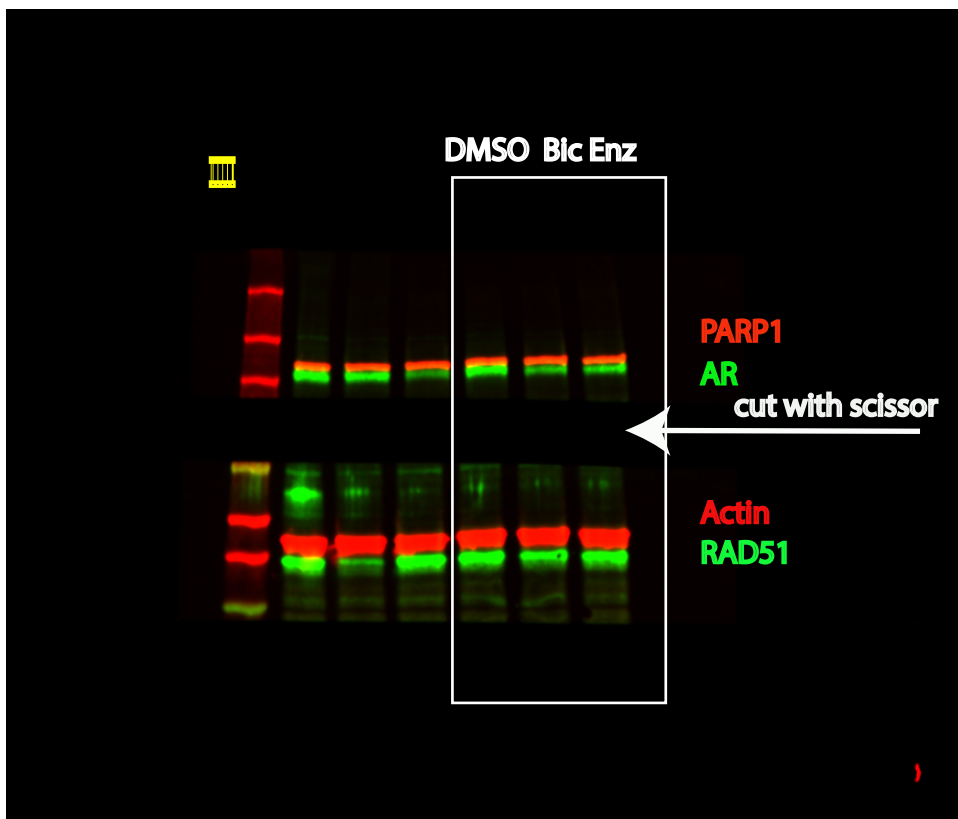
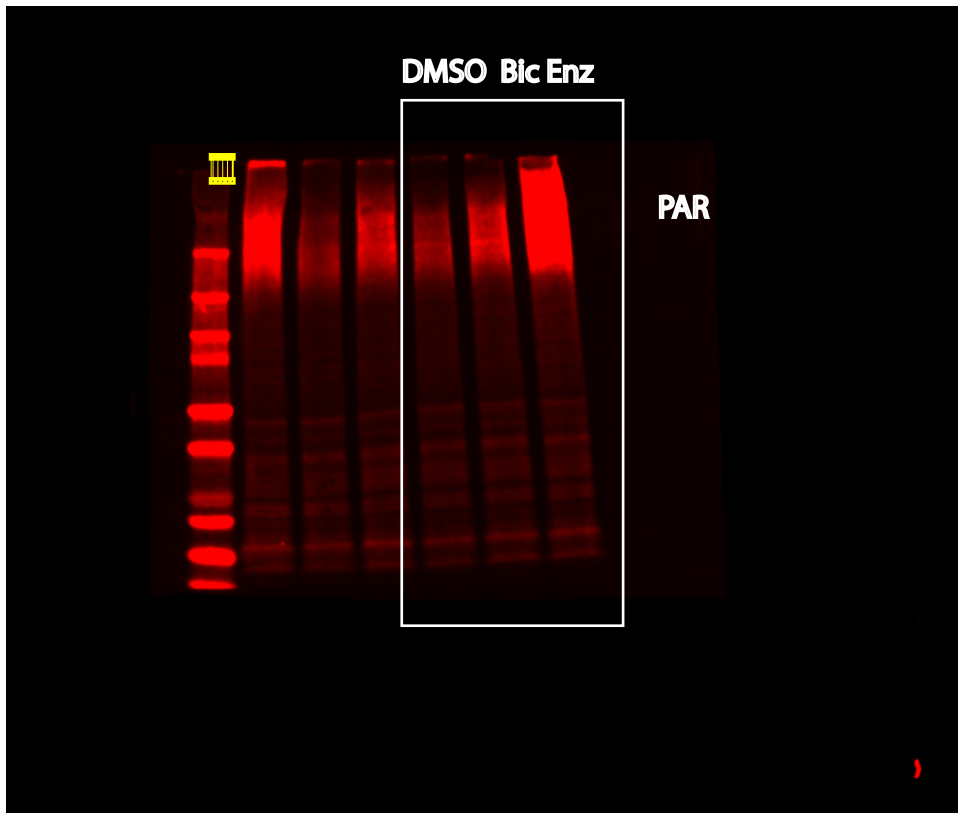
**Supplementary Figure 4. Effect of castration on PARP1 mRNA levels.** (a-b) PARP1 mRNA (red spots) expression in a fine-needle prostate biopsy taken from one patient before (a) and after (b) castration. The z-projection of the maximal intensity of 5 consecutive focal planes is shown. Scale bar, 20  $\mu\text{m}$ . (c) Quantification of the density of PARP1 transcripts in samples derived from 3 patients before and after castration. Whiskers, 5–95 percentile range. Grey dots, outliers.  $n$ , number of pseudo-cells with  $\geq 3$  mRNA analyzed in each patient. \*\*\* =  $p < 0.001$  (Mann Whitney). (d) Effects of castration in PARP1 protein levels. The fluorescence of PARP1 antibody connected fluorophores has been quantified in the same patients and biopsies as in c. For details, see material and methods. \* =  $p < 0.05$  (Mann Whitney).



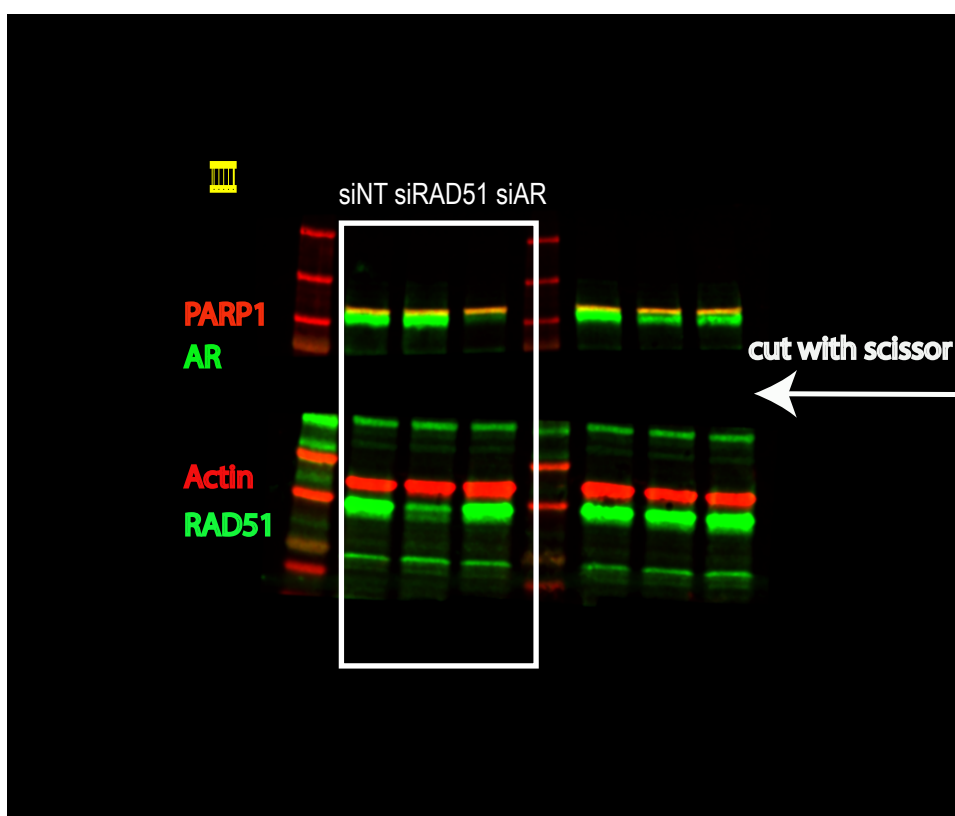
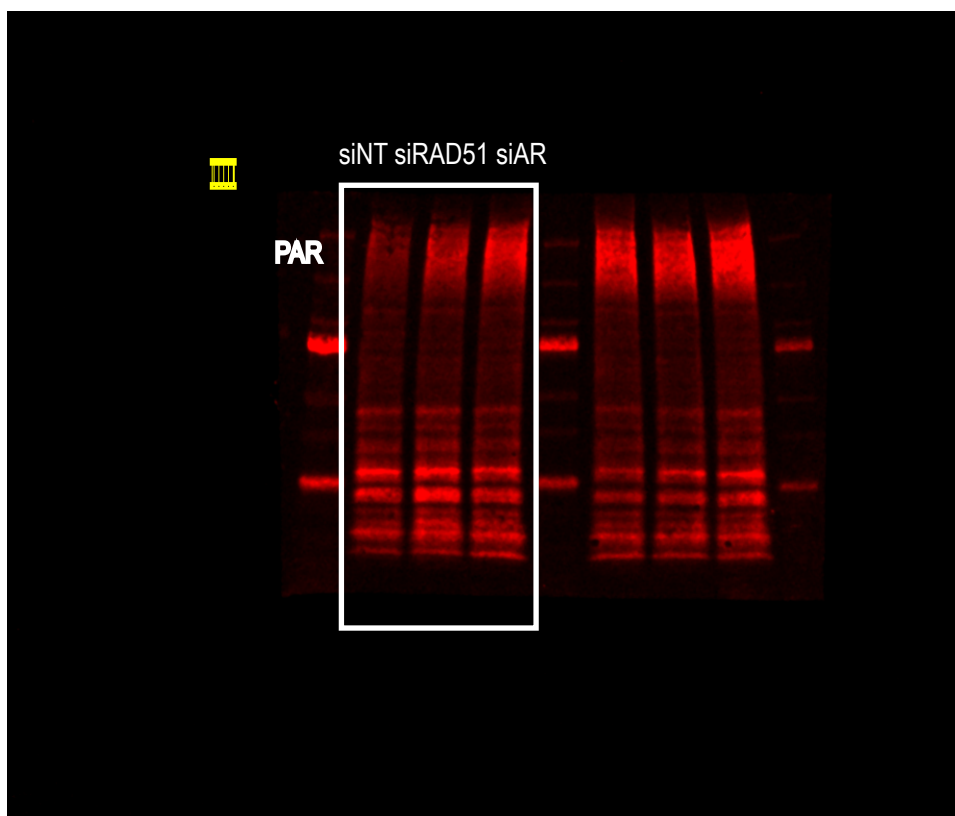
**Supplementary Figure 5. Effect of PARP inhibitor and ADT on the growth of prostate cancer cell lines.** (a, b) Viable fraction assessed by MTS assay of DuCaP (a) and (b) PNT1A cell lines treated with the indicated doses of Olaparib and/or enzalutamide or bicalutamide (10  $\mu$ M) for 7 days or until 95% confluence was reached; bars show mean  $\pm$ SEM. (c and d) Clonogenic cell survival assay for DuCaP (d) and LN3 (c) cells treated with bicalutamide or Enzalutamide (10  $\mu$ M) and/or Olaparib (1  $\mu$ M), P value by two-sided Student's t-test; bars show mean  $\pm$ SEM. (e and f) Viable fraction of PC3-T878A AR (e) and C4-2 (f) cells transfected with control (siINT) and siAR and treated with the indicated doses of Olaparib for 5 days; P value by two-sided Student's t-test. (g) MTS assay results showing the viable fractions of C4-2 cells transfected with non-targeting control, AR or PARP1 siRNA and treated with Olaparib (1  $\mu$ M) for 5 days; P value by two-sided Student's t-test.



**Supplementary Figure 6. Effect of PARP inhibitor and ADT on tumour xenograft and host animals.** (a) Tumour images of C4-2 xenograft in NSG mice treated with indicated drugs. (b) Body weight of C4-2 xenograft bearing mice treated with the indicated drugs. (c) Western blot of PC3 and PC3-AR cells showing the AR protein expression in each cell line,  $\beta$ -actin is used as internal control. (d and e) Tumour xenografts of PC3 (d) or PC3-AR (e) cell lines, NSG mice were administered with DMSO (vehicle) or Olaparib as indicated, P value by two-sided Student's t-test. (f) Body weight of mice bearing PC3 or PC3-AR xenografts and treated with indicated drugs. (g) Quantification of cleaved caspase-3 expression in ex vivo culture of human PCa treated with bicalutamide (10  $\mu$ M) and/or Olaparib (2  $\mu$ M) for 72 hr; significance calculated by Mann-Whitney U test. (h) Quantification of cleaved caspase-3 expression in ex vivo culture of human PCa treated with Enzalutamide (10  $\mu$ M) and/or Olaparib (2  $\mu$ M) for 72 hr, significance calculated by Mann-Whitney U test.



Supplementary Figure 7a: Uncropped Western blot image for figure 2a



Supplementary Figure 7b: Uncropped Western blot image for figure 2b

| Probe name | ENSEMBL gene    | ENSEMBL transcript | Oligo #  | Oligo sequence (5'->3') |
|------------|-----------------|--------------------|----------|-------------------------|
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_1  | TTATCCGAAGACTCCGCCAT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_2  | CTTGGCGTACTCGACTCGAT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_3  | ACTGCACCATGATGCCATC     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_4  | GGTACCAGTGTGGGACTTTT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_5  | ACCTTCCAGAAGCAGAGAA     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_6  | GAAGCTCAGAGAACCCTCC     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_7  | CAGCTTCCGCTGCTCTCTTG    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_8  | GAGTCTTCTCTGCCTTGCTA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_9  | ACTTGGCATACTCTGCTGCA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_10 | CCCTTTCGACGTACTTCTGT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_11 | ATCTTCTTGGACAGCGCAC     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_12 | TGGATGGTACCAGCGGTCAA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_13 | CAGCTCCTCCCTGTTCTTGA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_14 | AAGGAGGCTGAAGCCCTTGA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_15 | AGATTCTTCTTTCGCCACTT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_16 | GCTTTTAGGGCTTTTTCAG     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_17 | GATGTTCAGATCAGTCTGT     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_18 | ACACTTTCTTTAGCTCTGTC    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_19 | TGCTTGTGAAGATGAGTAG     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_20 | ATGCCATCAGTACTCGGTC     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_21 | TTGAAGACCAGCTGACCCGA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_22 | CCAGTGCAGTAATAGGCAT     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_23 | TGGGTGTCTGTGTTCTTGACC   |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_24 | TGAGGTAAGAGATTCTCTGG    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_25 | AGGAGTTTCACAGCAGCAGGA   |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_26 | GATAATGGCTTATCTGCTGA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_27 | TCCTTGTTCGGGACAGCTT     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_28 | AGTTTCTCAATCATGGCCTT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_29 | TGCACAGGGAAGCCTTGTGT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_30 | CCTCCATCTTCTTATTCATC    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_31 | CTCAGACACAACTCGGATGT    |
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| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_33 | GGACAGATGTGGCGCTAAGA    |
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| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_35 | GTTGATACCTTCCTCCTTGA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_36 | GAATCAGGATCCACAGCTGC    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_37 | GAAGACCTTCCACCTTTCT     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_38 | TTGTAGTAGGAGTTGGTTCC    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_39 | AGGACCTGAATATCCAATAC    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_40 | GGACGGCATCTGTCCAGTT     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_41 | GCGTTCCCGGTTTTTCTTCT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_42 | GGGGGTAGAACTTTTGGGA     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_43 | CATCCTGGCCATAGTCAATC    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_44 | CAAAGATCATCTTGATGAGG    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_45 | CTGAAGTCGATCTCATACT     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_46 | ATGGAGTATGGCGGCTGGAT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_47 | AGGATCTGAGAGTCGCTGCT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_48 | AGGGTGTAAGAGCATTTGA     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_49 | TTGTTACAGGACCGAGGCTT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_50 | CTCGATGTCCAGCAGGTTGT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_51 | GTTGACATCGATGGGATCCT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_52 | GGCTTCTCAGAATCTCTGT     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_53 | CCAAGTCATACGCAATCTGT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_54 | AACGCTGGCATTGCCTTCA     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_55 | ATGAAGCTGCTTAAAGGGCT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_56 | GATCCCAGCAAGTTGGTGG     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_57 | TACCAACATGTAGCCTGTC     |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_58 | GTTGGCACTCTTGGAGACCA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_59 | GCAACTTCTCCCAACAGGAT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_60 | TGGGTAACCTGCTGATATGT    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_61 | CAGGGGTAGTTTTGCCCCAA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_62 | CAAGAGGAACGTCTACACCA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_63 | CGTTATATAGTAGAGAGGTG    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_64 | CCTGAGCAATATCATAGACA    |
| PARP1      | ENSG00000143799 | ENST00000366794    | PARP1_65 | CAGGGAGGTCTTAAATTTGA    |

**Supplementary Table 1. List of oligo sequences used as smFISH probe targeting PARP1**

## Supplementary References

1. Grasso, C.S., *et al.* The mutational landscape of lethal castration-resistant prostate cancer. *Nature* **487**, 239-243 (2012).
2. Arredouani, M.S., *et al.* Identification of the transcription factor single-minded homologue 2 as a potential biomarker and immunotherapy target in prostate cancer. *Clin Cancer Res* **15**, 5794-5802 (2009).
3. LaTulippe, E., *et al.* Comprehensive gene expression analysis of prostate cancer reveals distinct transcriptional programs associated with metastatic disease. *Cancer Res* **62**, 4499-4506 (2002).