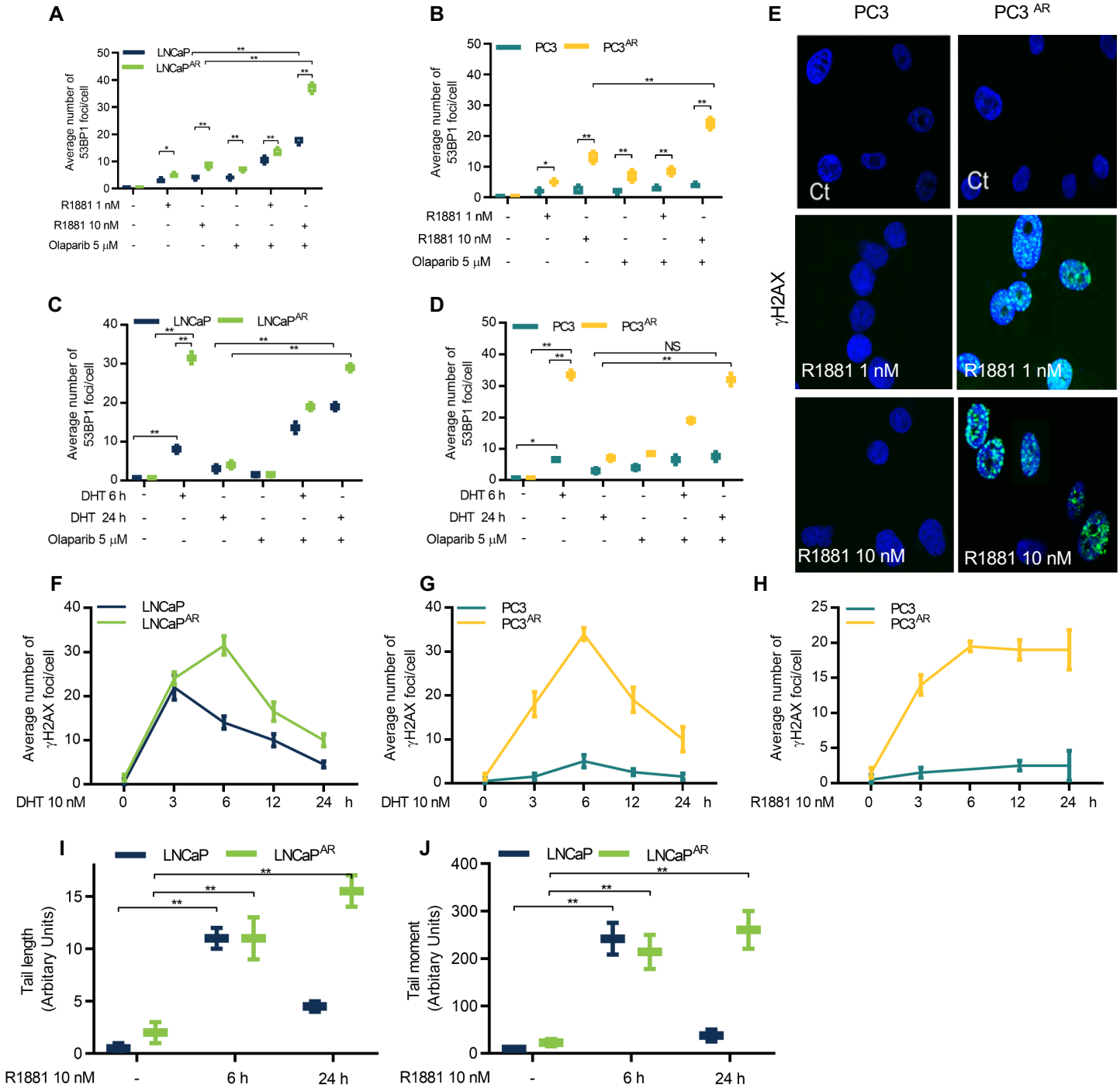


**Fig S1: Androgen induced DNA DSBs are dependent on AR. (A,C)** Quantitation of 53BP1 foci following exposure to R1881, 10 nM DHT and olaparib alone or in combination for 24 h in LNCaP, LNCaP$^{AR}$ **(B,D)** Quantitation of 53BP1 foci following exposure to R1881, 10 nM DHT and olaparib alone or in combination for 24 h in PC3 and PC3$^{AR}$ cells respectively. **(E)** Confocal immunostaining of γH2AX in PC3 and PC3$^{AR}$ cells treated with different concentrations of R1881. **(F,G,H)** Quantitation of the kinetics of DNA damage by yH2AX foci/cell in LNCaP, LNCaP$^{AR}$, and PC3 and PC3$^{AR}$ following 10 nM R1881 or DHT at intervals after treatment. **(I,J)** Graphs represent comet tail moment and length in LNCaP and LNCaP$^{AR}$ cells following 10 nM R1881. In A-D, and F-I, data represent the mean ± SD; n=4 replicates per experiment; $p \leq 0.05$ (*), $p < 0.01$ (**) by two-way ANOVA.

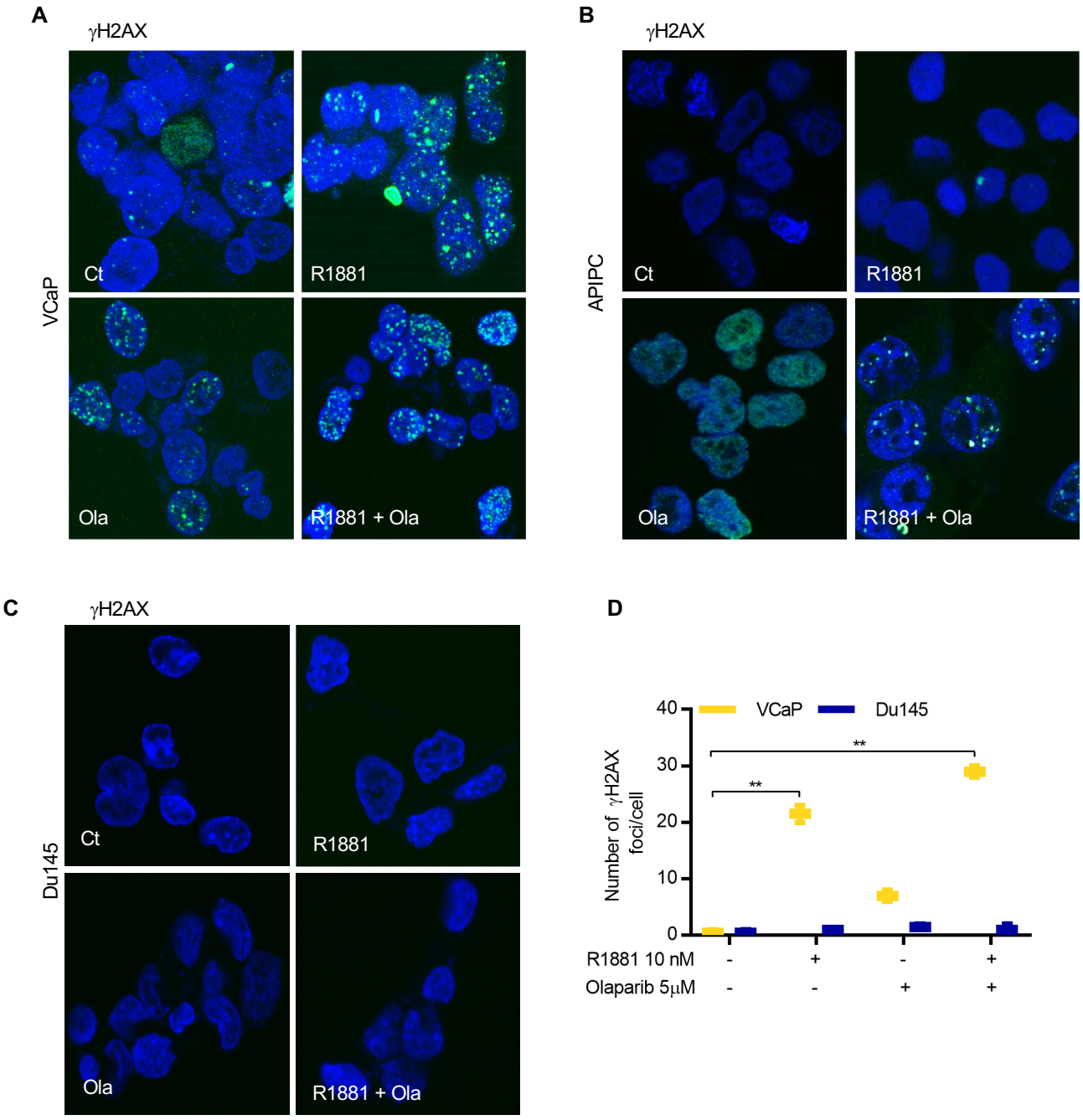


**Fig S2: Assessments of DNA damage in AR negative and positive cells following SPA and Olaparib Treatment. (A,B,C)** Confocal immunostaining of γH2AX in VCaP, LNCaP$^{APIPC}$ and Du145 cells treated with 10 nM R1881 and Olaparib (Ola) alone or in combination. **(D)** Graphical representation of γH2AX in VCaP and Du145 cells treated with 10 nM R1881 and Olaparib alone or in combination. ** $p<0.01$ by two-way ANOVA and n=3 experimental replicates.

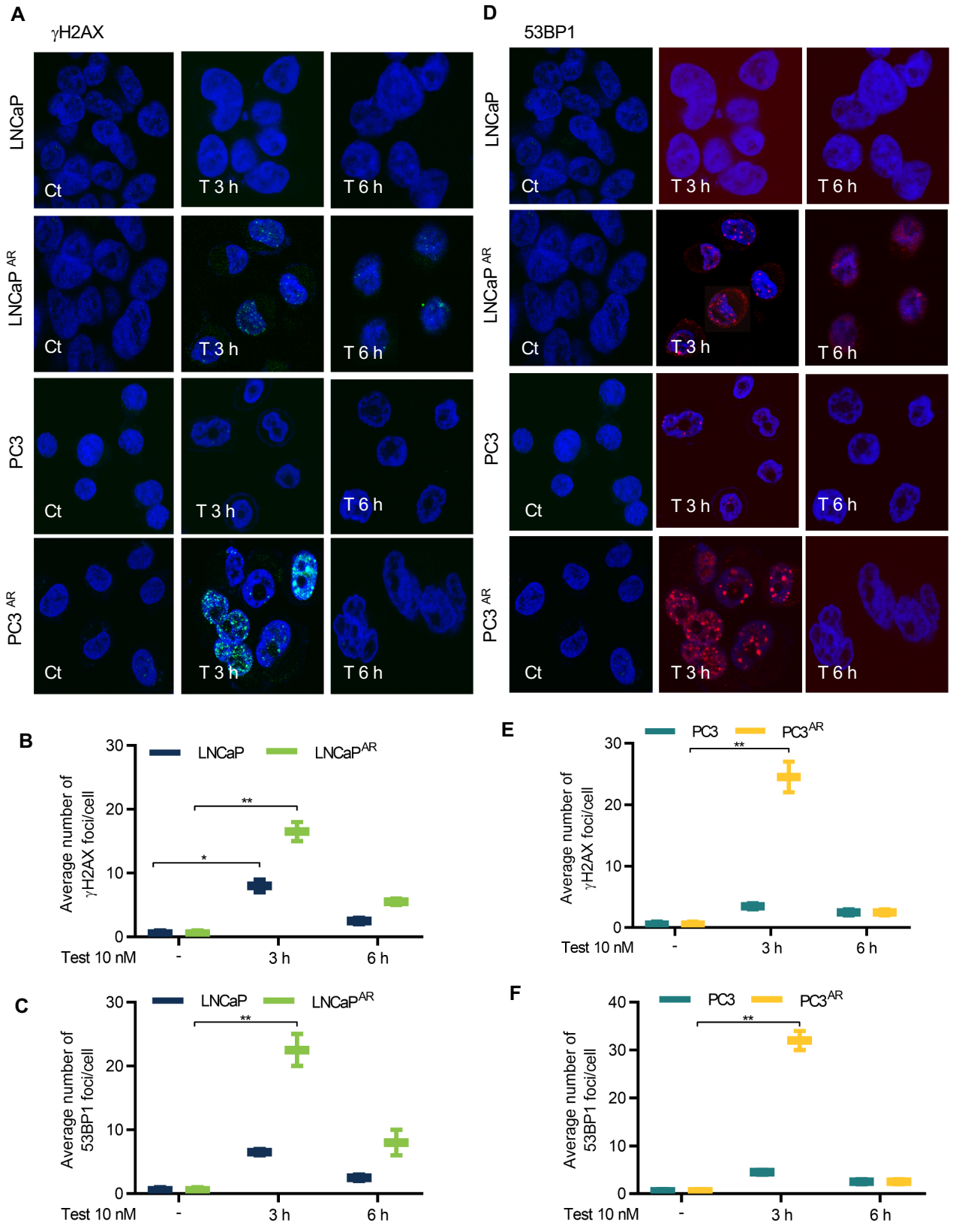


***Fig S3: Kinetics of testosterone induced DNA DSBs.*** Confocal immunostaining (**A,D**) and graphical representation (**B,E**) of γH2AX and (**C,F**) 53BP1 in LNCaP, LNCaP$^{AR}$, and PC3 and PC3$^{AR}$ respectively following 10 nM testosterone exposure for 3 and 6h. In B,C,E,F data represent the mean ± SD; n=4 replicates per experiment; p≤0.05 (*), p<0.01 (**) by two-way ANOVA.

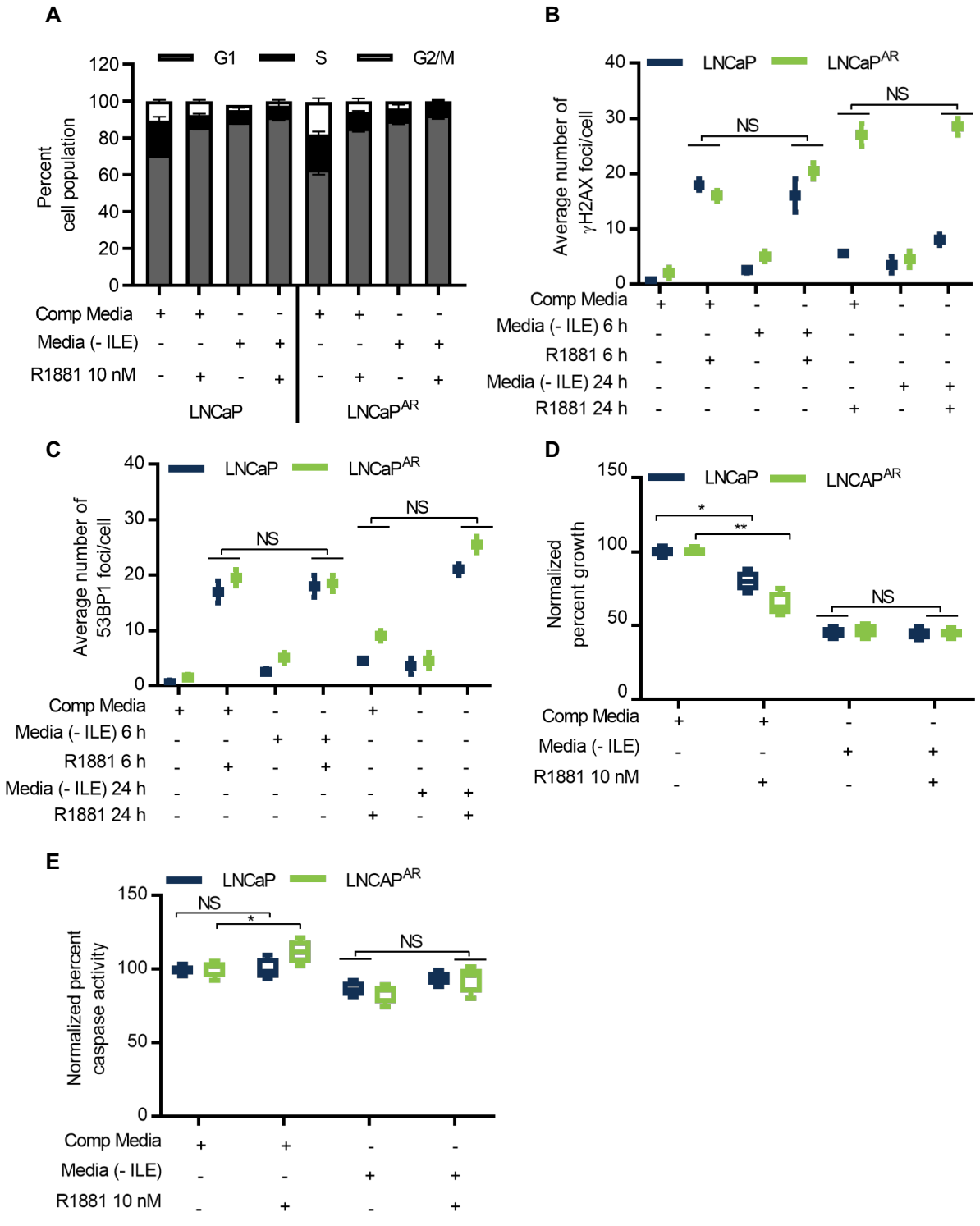


***Fig S4: SPA induces DNA damage in quiescent prostate cancer cells.*** **(A)** Cell cycle analysis demonstrates the synchronizing effect of isoleucine starvation in both LNCaP and LNCaP$^{AR}$ cells. **(B,C)** Graphical representation of yH2AX and 53BP1 foci in LNCaP and LNCaP$^{AR}$ cells following SPA treatment under isoleucine deprived condition (- ILE). **(D,E)** Percentage of cell growth and caspase activity was evaluated after 3 days of R1881 exposure under the isoleucine depleted conditions * $p \leq 0.05$, ** $p < 0.01$ by two way ANOVA and n=4 replicates.

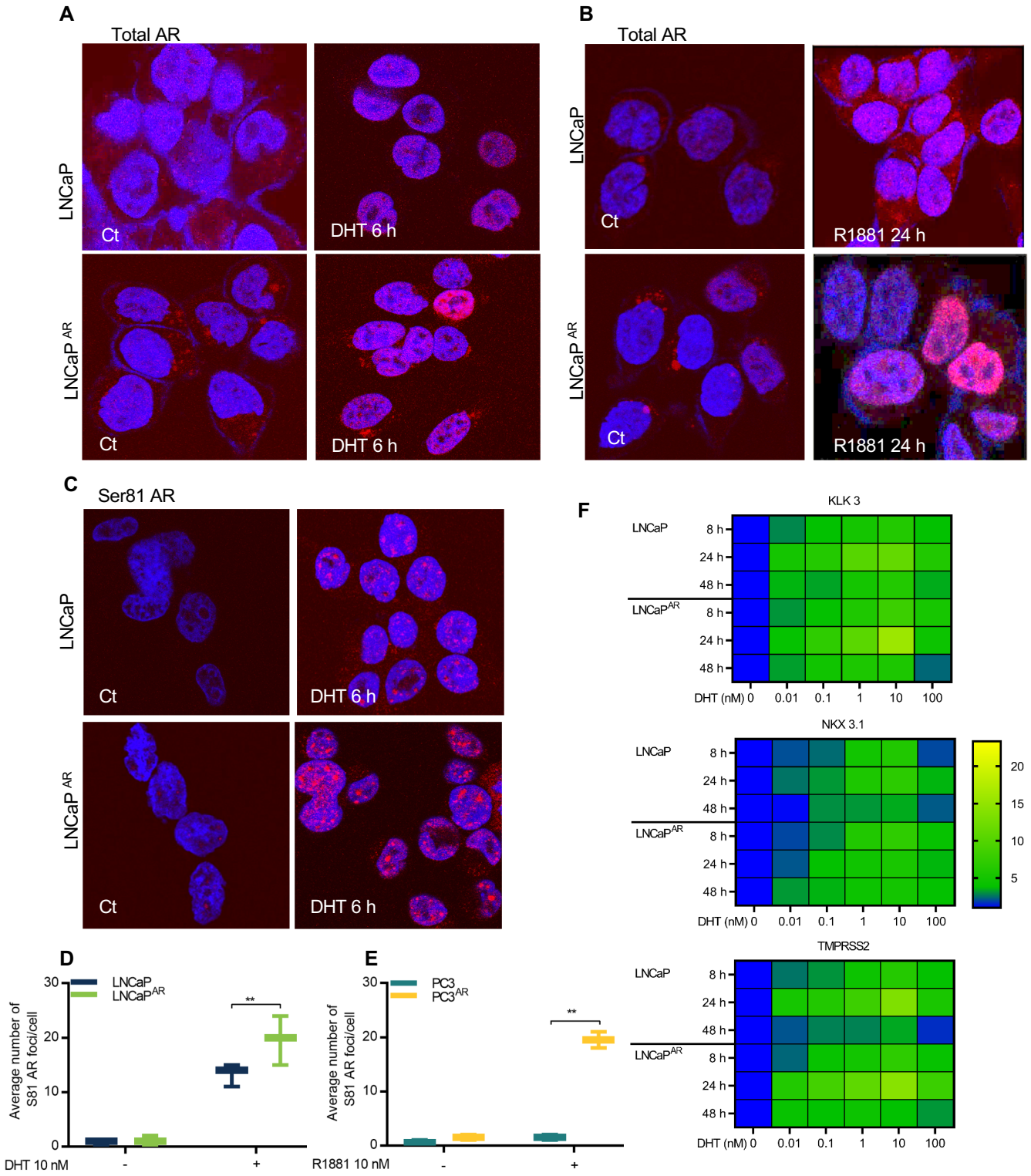


**Fig S5: Effects of SPA on AR localization and function.** Confocal immunostaining of total AR in LNCaP and LNCaP$^{AR}$ cells following **(A)** 10 nM DHT and **(B)** 10 nM R1881**. (C,D)** Confocal immunostaining and graphical representation of Ser81 AR in LNCaP and LNCaP$^{AR}$ cells following 10 nM DHT. **(E)** Quantitation of Ser81 AR foci in PC3 and PC3AR after 24h of 10 nM R1881 treatment. **(F)** Transcript levels of KLK3, NKX3.1 and TMPRSS2 in LNCaP cells following exposure to DHT concentrations 0.01 to 100 nM. ** p<0.01 by two way ANOVA and n=4 replicates.

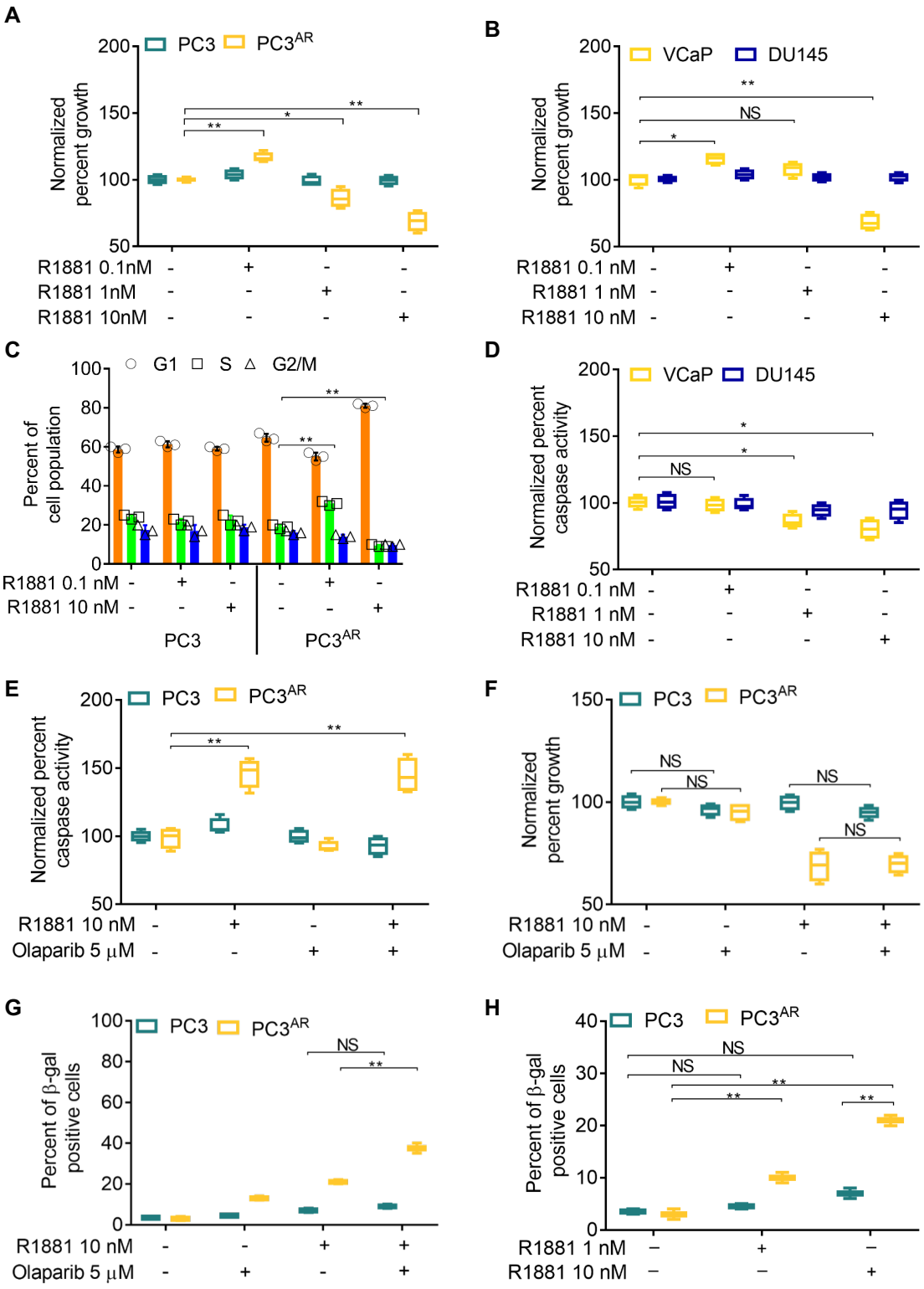
A
PC3
PC3AR
Normalized percent growth
200
150
100
50
**
*
**
R1881 0.1nM - + - -
R1881 1nM - - + -
R1881 10nM - - - +
B
VCaP
DU145
Normalized percent growth
200
150
100
50
**
NS
*
R1881 0.1 nM - + - -
R1881 1 nM - - + -
R1881 10 nM - - - +
C
G1
S
G2/M
Percent of cell population
100
80
60
40
20
0
**
**
R1881 0.1 nM - + - - + -
R1881 10 nM - - + - - +
PC3
PC3AR
D
VCaP
DU145
Normalized percent caspase activity
200
150
100
50
*
*
NS
R1881 0.1 nM - + - -
R1881 1 nM - - + -
R1881 10 nM - - - +
E
PC3
PC3AR
Normalized percent caspase activity
200
150
100
50
**
**
R1881 10 nM - + - +
Olaparib 5 μM - - + +
F
PC3
PC3AR
Normalized percent growth
150
100
50
NS
NS
NS
NS
R1881 10 nM - - + +
Olaparib 5 μM - + - +
G
PC3
PC3AR
Percent of β-gal positive cells
100
80
60
40
20
0
NS
**
R1881 10 nM - - + +
Olaparib 5 μM - + - +
H
PC3
PC3AR
Percent of β-gal positive cells
40
30
20
10
0
NS
NS
**
**
**
R1881 1 nM – + –
R1881 10 nM – – +

**Fig S6: Effects of SPA and PARP inhibition on prostate cancer cell proliferation, apoptosis and senescence. (A,B)** Effects of androgen concentrations on the growth of PC3, $PC3^{AR}$ VCaP and DU145 cells after 3 days. **(C)** Cell cycle profile for PC3 and $PC3^{AR}$ following different concentrations of R1881 for 3 days. **(D, E)** Quantitation of apoptosis by caspase activity in VCaP and Du145 and PC3 and $PC3^{AR}$ cells following exposure to R1881 and Olaparib for 3 days. **(F,G,H)** Quantitation of cell growth and senescence by senescence-associated beta-galactosidase staining. The percentage of senescent cells is shown after 3 days treatment of different doses of R1881 and olaparib in PC3 and $PC3^{AR}$ cells. Data represent the mean ± SD; n=4 replicates per experiment; $p \leq 0.05$ (*), $p < 0.01$ (**) by two-way ANOVA.

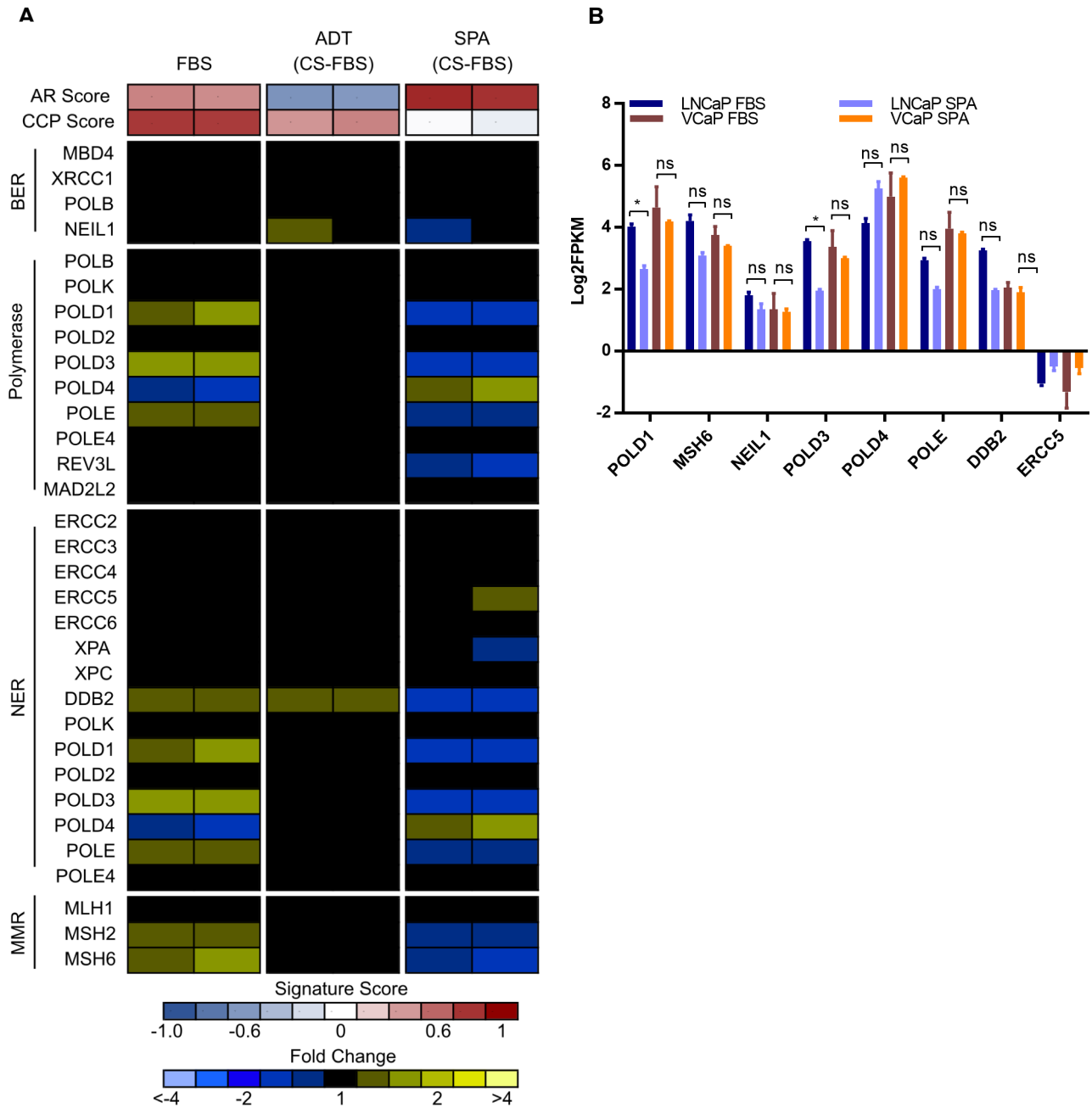


***Fig S7. Involvement of other repair pathways such as BER, NER and MMR along with polymerases, in LNCaP cells following SPA treatment.*** **A.** Transcript levels of BER, NER, MMR and polymerase components determined by RNAseq analysis in LNCaP cells in standard growth medium (FBS) or in androgen depleted medium, ADT (CS-FBS), or androgen depleted medium supplemented with 10 nM R1881, SPA. **B.** graphical representation of the changes in transcript levels of the repair factors following SPA treatment compared to FBS.

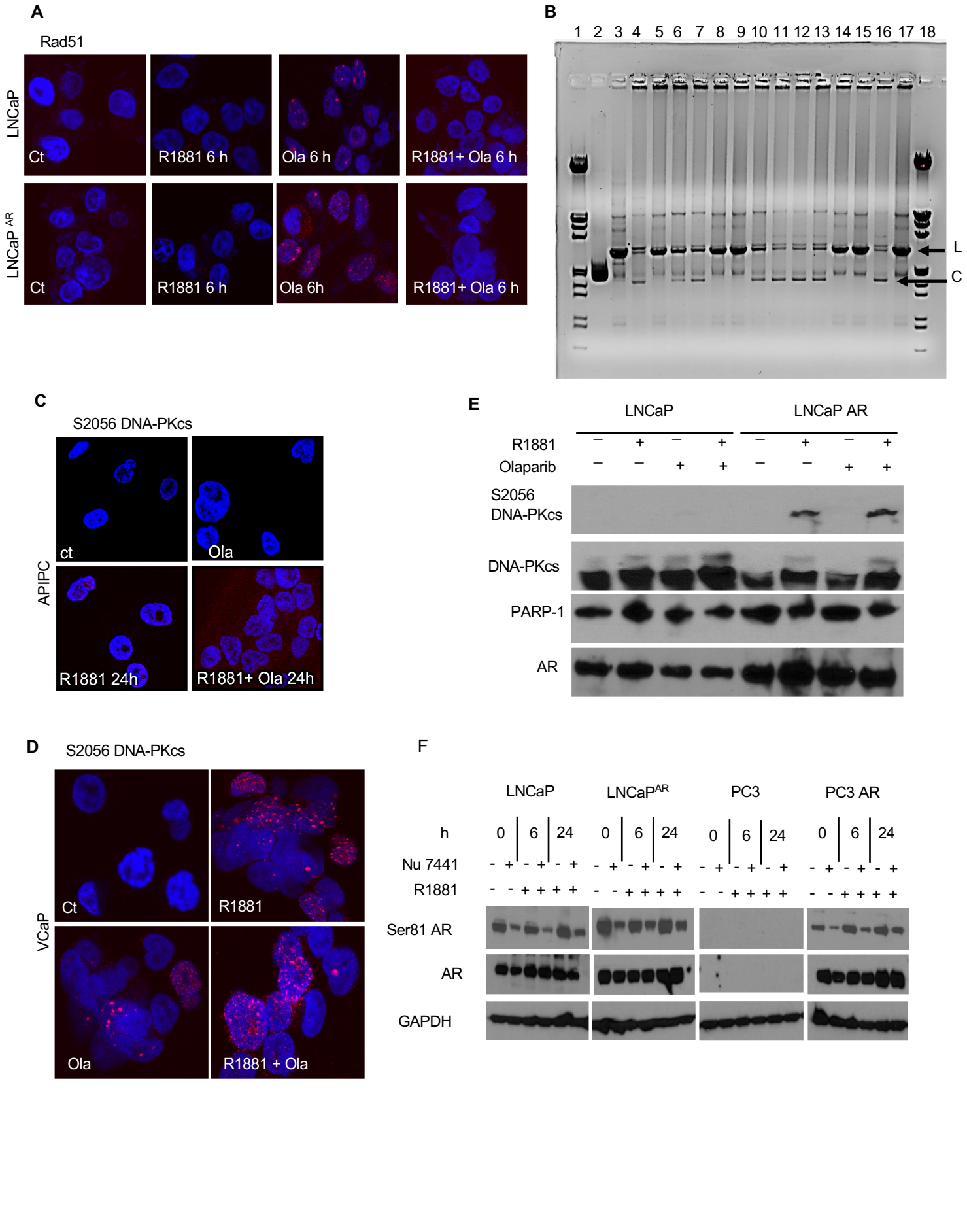
A
Rad51
LNCaP
Ct
R1881 6 h
Ola 6 h
R1881+ Ola 6 h
LNCaP AR
Ct
R1881 6 h
Ola 6h
R1881+ Ola 6 h
B
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
L
C
C
S2056 DNA-PKcs
APIPC
ct
Ola
R1881 24h
R1881+ Ola 24h
E
LNCaP
LNCaP AR
R1881
Olaparib
S2056 DNA-PKcs
DNA-PKcs
PARP-1
AR
D
S2056 DNA-PKcs
VCaP
Ct
R1881
Ola
R1881 + Ola
F
LNCaP
LNCaP^AR
PC3
PC3 AR
h
0 6 24
Nu 7441
R1881
Ser81 AR
AR
GAPDH

**Fig S8: Assessments of HR- and NHEJ-DNA repair pathways following SPA treatment in prostate cancer cells. (A)** Confocal immunostaining of RAD51, a marker of HR repair in both LNCaP and LNCaP$^{AR}$ cells following SPA alone and Olaparib treatment in combination **(B)** In-vitro DNA end ligation assay, a surrogate experiment to evaluate for NHEJ repair assay using the nuclear extract of all prostate cancer cells LNCaP, LNCaP$^{AR}$ and PC3, PC3$^{AR}$ cells treated with SPA on pUC19 DNA. Lane 1 and 18 are lambda phage markers. Lane 2 is circular pUC19 DNA (C), Lane 3 is EcoR1 restriction digested linearized pUC19 DNA (L), Lane 4-6 are LNCaP cells' nuclear extract treated DNA samples (untreated, 6 and 24h of 10 nM R1881 respectively), Lanes 7-9 are LNCaP$^{AR}$ cells' nuclear extract treated DNA samples (untreated, 6 and 24h of 10 nM R1881 respectively), lanes 10-12 and lanes 13-15 are PC3 and PC3$^{AR}$ cells' nuclear extract treated DNA samples (untreated, 6 and 24h of 10 nM R1881 respectively). Lane 16 is T4 DNA ligase treated pUC19 DNA, used as positive control and lane 17 is pUC19 DNA with no T4 DNA ligase used as negative control. ***(*C) Confocal immunostaining of Ser2056 DNA-PKcs foci in LNCaP$^{APIPC}$ cells treated with SPA or olaparib (Ola) or in combination. **(D)** Confocal immunostaining of Ser2056 DNA-PKcs foci in VCaP cells treated with SPA or olaparib or in combination. **(E)** Chromatin fractionation assay in LNCaP and LNCaP$^{AR}$ cells treated with SPA, olaparib and in combination and probed for Ser 2056 DNA-PKcs, DNA-PKcs, PARP-1 and AR. **(F)** Western blot probed for Ser81 AR, AR and GAPDH in LNCaP, LNCaP$^{AR}$ and PC3, PC3$^{AR}$ cells treated with SPA and DNA-PKcs inhibitor NU7441 alone or in combination

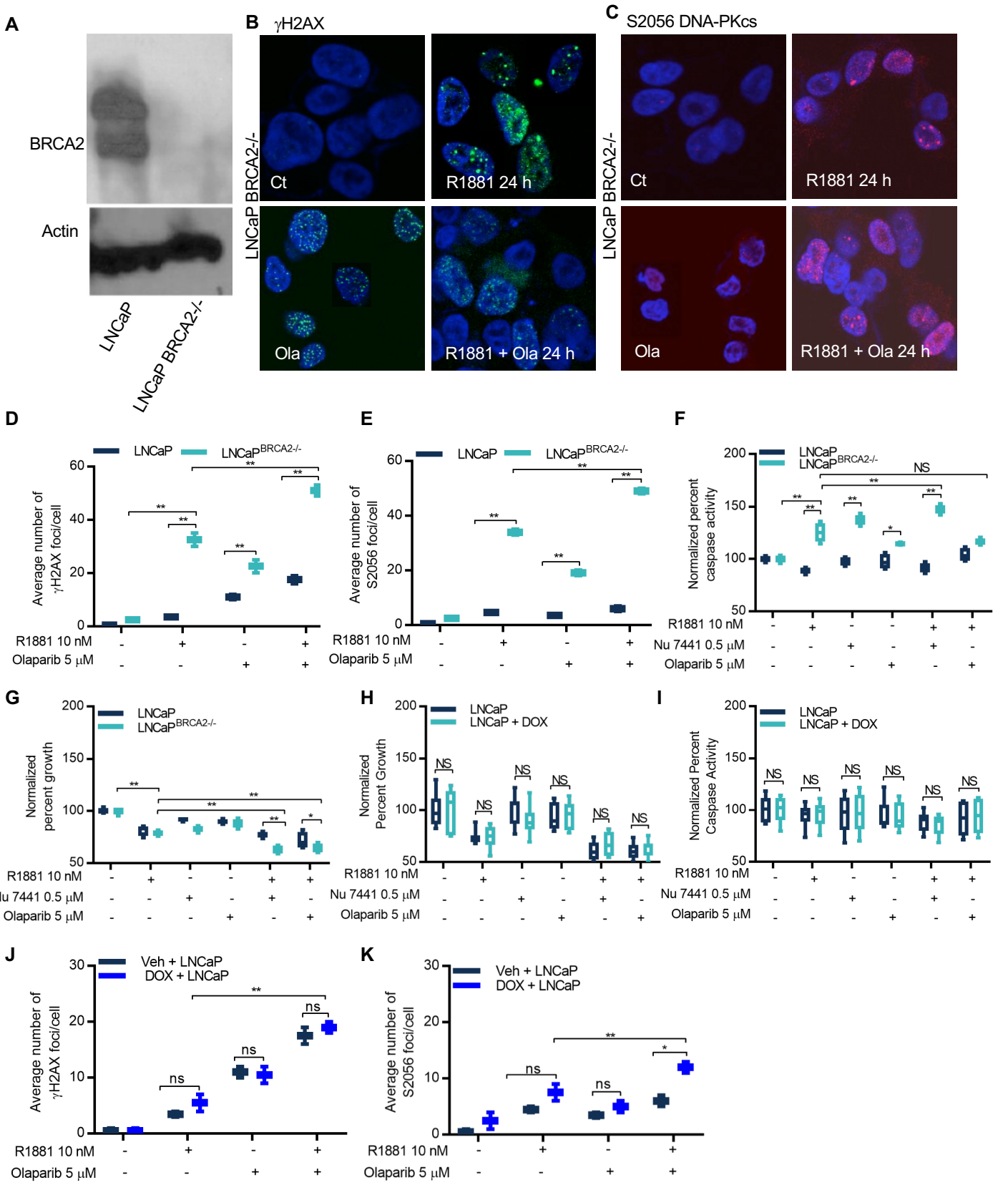
A
BRCA2
Actin
LNCaP
LNCaP BRCA2-/-
B
γH2AX
LNCaP BRCA2-/-
Ct
R1881 24 h
LNCaP
Ola
R1881 + Ola 24 h
C
S2056 DNA-PKcs
LNCaP BRCA2-/-
Ct
R1881 24 h
LNCaP
Ola
R1881 + Ola 24 h
D
LNCaP
LNCaP BRCA2-/-
Average number of γH2AX foci/cell
R1881 10 nM
Olaparib 5 μM
E
LNCaP
LNCaP BRCA2-/-
Average number of S2056 foci/cell
R1881 10 nM
Olaparib 5 μM
F
LNCaP
LNCaP BRCA2-/-
Normalized percent caspase activity
R1881 10 nM
Nu 7441 0.5 μM
Olaparib 5 μM
G
LNCaP
LNCaP BRCA2-/-
Normalized percent growth
R1881 10 nM
Nu 7441 0.5 μM
Olaparib 5 μM
H
LNCaP
LNCaP + DOX
Normalized Percent Growth
R1881 10 nM
Nu 7441 0.5 μM
Olaparib 5 μM
I
LNCaP
LNCaP + DOX
Normalized Percent Caspase Activity
R1881 10 nM
Nu 7441 0.5 μM
Olaparib 5 μM
J
Veh + LNCaP
DOX + LNCaP
Average number of γH2AX foci/cell
R1881 10 nM
Olaparib 5 μM
K
Veh + LNCaP
DOX + LNCaP
Average number of S2056 foci/cell
R1881 10 nM
Olaparib 5 μM

***Fig S9. Evaluating the effects of SPA and Olaparib in a LNCaP BRCA2 CRISPR model.*** **A.** Western immunoblot of BRCA2 protein extracts from LNCaP and LNCaP with CRISPR/Cas9 *BRCA2* gene editing. **B.** Confocal immunostaining and **D**. quantitation of yH2AX in LNCaP *BRCA2-/-* cells following 10 nM R1881 and/or Olaparib treatment for 24h. **C.** Confocal immunostaining and **E**. quantitation of DNA PKcs S2056 foci in LNCaP *BRCA2*-/- cells following 10 nM R1881 and/or Olaparib treatment for 24h. **F**. Quantitation of apoptosis by caspase activity, and **G**. growth of LNCaP *BRCA2-/-* cells after 3 days treatment with R1881, Nu7441 or Olaparib. **H. and I.** Graphical representation of the estimated growth and caspase activity in LNCaP under the presence or absence of DOX after 3 days treatment with R1881, Nu7441 or Olaparib. **J.** and **K**. Quantitation of yH2AX foci and Ser2056foci in LNCaP cells in the presence or absence of DOX following treatment with R1881or Olaparib. In D, E, F, G, I, J and K data represent mean ± SD; n=4 replicates per experiment; $p \leq 0.05$ (*), $p < 0.01$ (**) by two-way ANOVA.

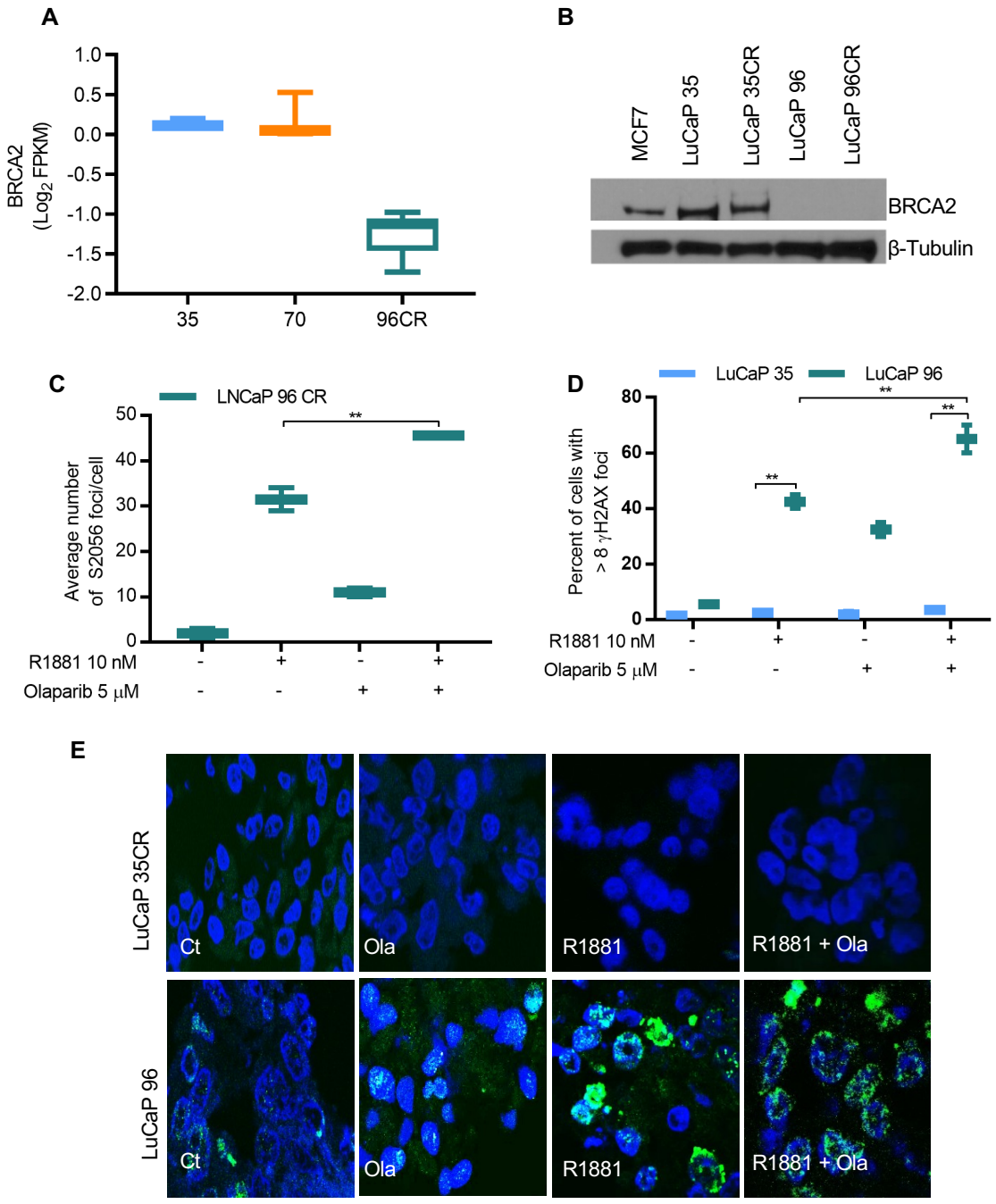


***Fig S10. Assessment of the effects of SPA and olaparib in LuCaP PDX models with and without BRCA2 Loss:*** **(A)** Transcript levels of BRCA2 in LuCaP 35, LuCaP 70 and LuCaP 96CR PDX tumors determined by RNAseq and plotted as the Log2 of fragments per kilobase of transcript per million mapped reads (FPKM); **(B)** Western blot (3-8% Tris-Acetate Gel) probed for BRCA2 protein in MCF7 breast cancer cells as a positive control, and LuCaP 35, LuCaP 35CR, LuCaP 96 and LuCaP 96CR PDX lines. The approximate molecular weight of the BRCA2 band is 400 kDa; **(C)** Graphs represent the ser2056 DNA-PKcs foci in LuCaP 96CR following R1881 and/or olaparib after 18h exposure; **(D)** Graphs and **(E)** confocal immunostaining of yH2AX staining in LuCaP 35CR and LuCaP 96 tissue slice cultures treated *ex-vivo* for 3 days with R1881 and/or olaparib. Data represent the mean ± SD; n=3 replicates per experiment; $p \leq 0.05$ (*), $p < 0.01$ (**) by two-way ANOVA.

**Table S1. DNA repair gene mutations and PSA responses in men with CRPC enrolled on clinical trials of supraphysiological androgen therapy.**

| Patient ID | Homologous Recombination (HR) Gene Mutation | HR Mutation | PSA decline from baseline (%) | Sequencing Platform | Specimen Tested |
|---|---|---|---|---|---|
| 1 | BRCA2 S1982Rfs*22 | Yes | -100% | Color | Germline DNA |
| 2 | BRCA2 p.R2991C | Yes | -99% | PlasmaSELECT | Plasma |
| 3 | ATM R2244Kfs*5 | Yes | -98% | PlasmaSELECT | Tumor Tissue |
| 4 | BRCA2 N319Kfs*8 | Yes | -98% | Color | Germline DNA |
| 5 | ATM p.V1197Ffs*10 | Yes | -91% | UW-OncoPlex | Plasma |
| 6 | 1) CHD1 copy loss<br>2) Chromosome 13 copy loss in the region of BRCA2 | Yes | -88% | UW-OncoPlex | Plasma |
| 7 | 1) CDK12 p.G923V mutation and copy neutral LOH<br>2) BRCA2 p.Y1762* mutation and 1 copy loss | Yes | -84% | UW-OncoPlex | Plasma |
| 8 | ATM splicing mutation with LOH | Yes | -78% | UW-OncoPlex | Plasma |
| 9 | CHEK2 p.I157T | Yes | -74% | UW-OncoPlex | Plasma |
| 10 | CHEK2 p.I157T | Yes | -68% | UW-OncoPlex | Plasma |
| 11 | CHD1 copy loss | Yes | -67% | UW-OncoPlex | Plasma |
| 12 | 1) BRCA1 c.1378_1381del<br>2) CHEK2 p.I157T<br>3) CDK12 c.366dup | Yes | -61% | UW-OncoPlex | Plasma |
| 13 | PALB2 Q1164* | Yes | -61% | PlasmaSELECT | Tumor Tissue |
| 14 | CHEK2 p.L348Kfs*20 | Yes | -55% | UW-OncoPlex | Plasma |
| 15 | ATM R805* | Yes | -54% | FoundationOne | Tumor Tissue |
| 16 | FANCD2 S64Rfs*4 | Yes | -44% | FoundationOne | Tumor Tissue |
| 17 | PALB2 Q60Rfs*7 | Yes | -40% | PlasmaSELECT | Tumor Tissue |
| 18 | BRCA2 S1982Rfs*22 | Yes | -18% | FoundationOne | Tumor Tissue |
| 19 | BRCA2 copy loss | Yes | -11% | SU2C | Tumor Tissue |
| 20 | ATM p.Q1627X and p.L2427R | Yes | 0% | UW-OncoPlex | Plasma |
| 21 | ATM c.8876_8879del | Yes | 35% | UW-OncoPlex | Plasma |
| 22 | FANCA p.R853* | Yes | 37% | UW-OncoPlex | Plasma |
| 23 | BRCA2 p.Y1655X mutation and LOH | Yes | 130% | UW-OncoPlex | Plasma |
| 24 | BRCA2 S2371ks*21 | Yes | 253% | FoundationOne | Tumor Tissue |
| 25 | PALB2 p.W1038X | Yes | 310% | UW-OncoPlex | Plasma |
| 26 | BRCA2 p.Y2977Ffs*11 | Yes | 353% | UW-OncoPlex | Plasma |
| 27 | CHEK2 p.L348Kfs*20 | Yes | 513% | Color | Germline DNA |
| 28 | CDK12 c.1995dup | Yes | 603% | UW-OncoPlex | Plasma |
| 29 | ATM p.Q284X* | Yes | 662% | PlasmaSELECT | Plasma |
| 30 | | No | -94% | UW-OncoPlex | Tumor Tissue |
| 31 | | No | -85% | UW-OncoPlex | Tumor Tissue |
| 32 | | No | -72% | UW-OncoPlex | Plasma |
| 33 | | No | -66% | UW-OncoPlex | Plasma |

| | | | | | |
|---|---|---|---|---|---|
| 34 | | No | -63% | FoundationOne | Tumor Tissue |
| 35 | | No | -51% | UW-OncoPlex | Plasma |
| 36 | | No | -48% | FoundationOne | Tumor Tissue |
| 37 | | No | -38% | PlasmaSELECT | Tumor Tissue |
| 38 | | No | -36% | UW-OncoPlex | Plasma |
| 39 | | No | -35% | UW-OncoPlex | Plasma |
| 40 | | No | -11% | UW-OncoPlex | Plasma |
| 41 | | No | -8% | UW-OncoPlex | Plasma |
| 42 | | No | 13% | UW-OncoPlex | Plasma |
| 43 | | No | 22% | UW-OncoPlex | Plasma |
| 44 | | No | 26% | UW-OncoPlex | Plasma |
| 45 | | No | 28% | Guardant360 | Plasma |
| 46 | | No | 28% | UW-OncoPlex | Plasma |
| 47 | | No | 49% | UW-OncoPlex | Plasma |
| 48 | | No | 65% | UW-OncoPlex | Plasma |
| 49 | | No | 81% | UW-OncoPlex | Plasma |
| 50 | | No | 83% | FoundationOne | Tumor Tissue |
| 51 | | No | 122% | UW-OncoPlex | Plasma |
| 52 | | No | 135% | PlasmaSELECT | Tumor Tissue |
| 53 | | No | 144% | UW-OncoPlex | Plasma |
| 54 | | No | 154% | FoundationOne | Tumor Tissue |
| 55 | | No | 158% | PlasmaSELECT | Plasma |
| 56 | | No | 424% | UW-OncoPlex | Plasma |
| 57 | | No | 494% | FoundationOne | Tumor Tissue |
| 58 | | No | 514% | UW-OncoPlex | Plasma |
| 59 | | No | 529% | FoundationOne | Tumor Tissue |
| 60 | | No | 785% | PlasmaSELECT | Plasma |
| 61 | | No | 2027% | PlasmaSELECT | Tumor Tissue |
| 62 | | No | 2147% | FoundationOne | Tumor Tissue |