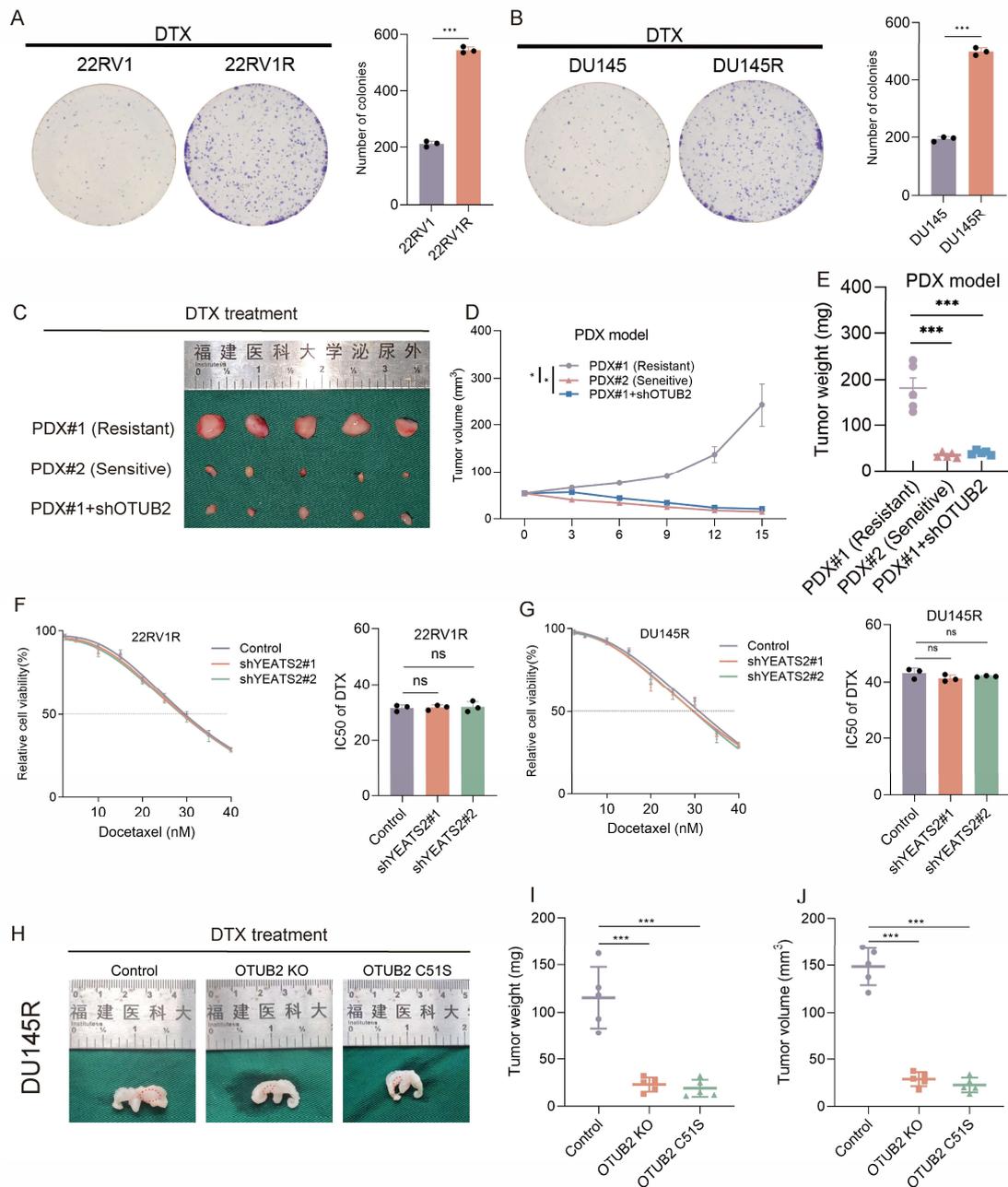


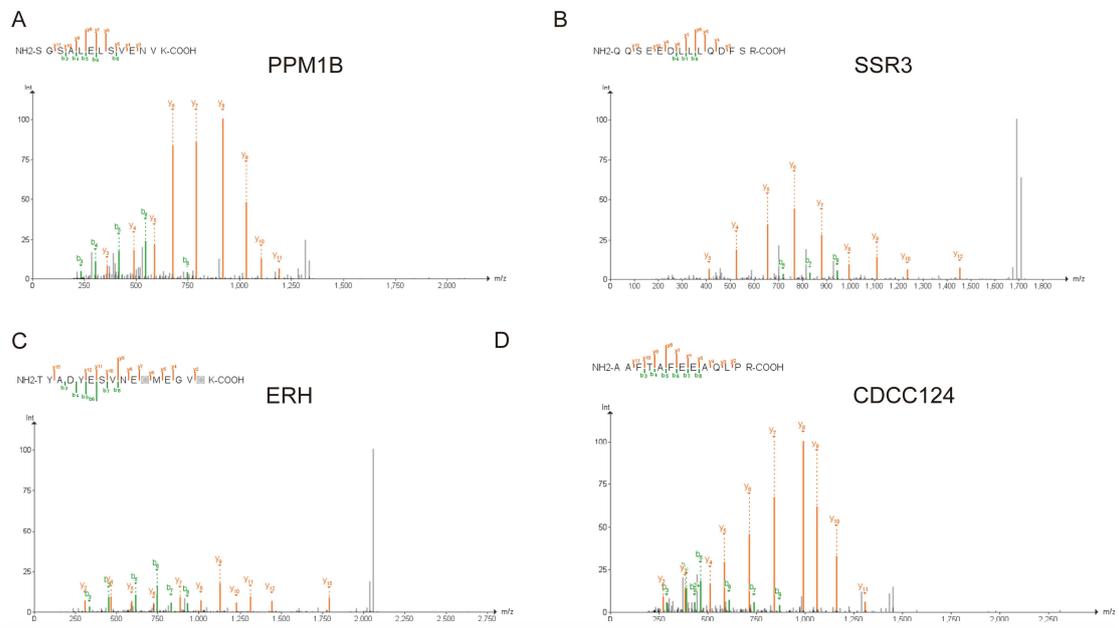
## Supplementary Materials



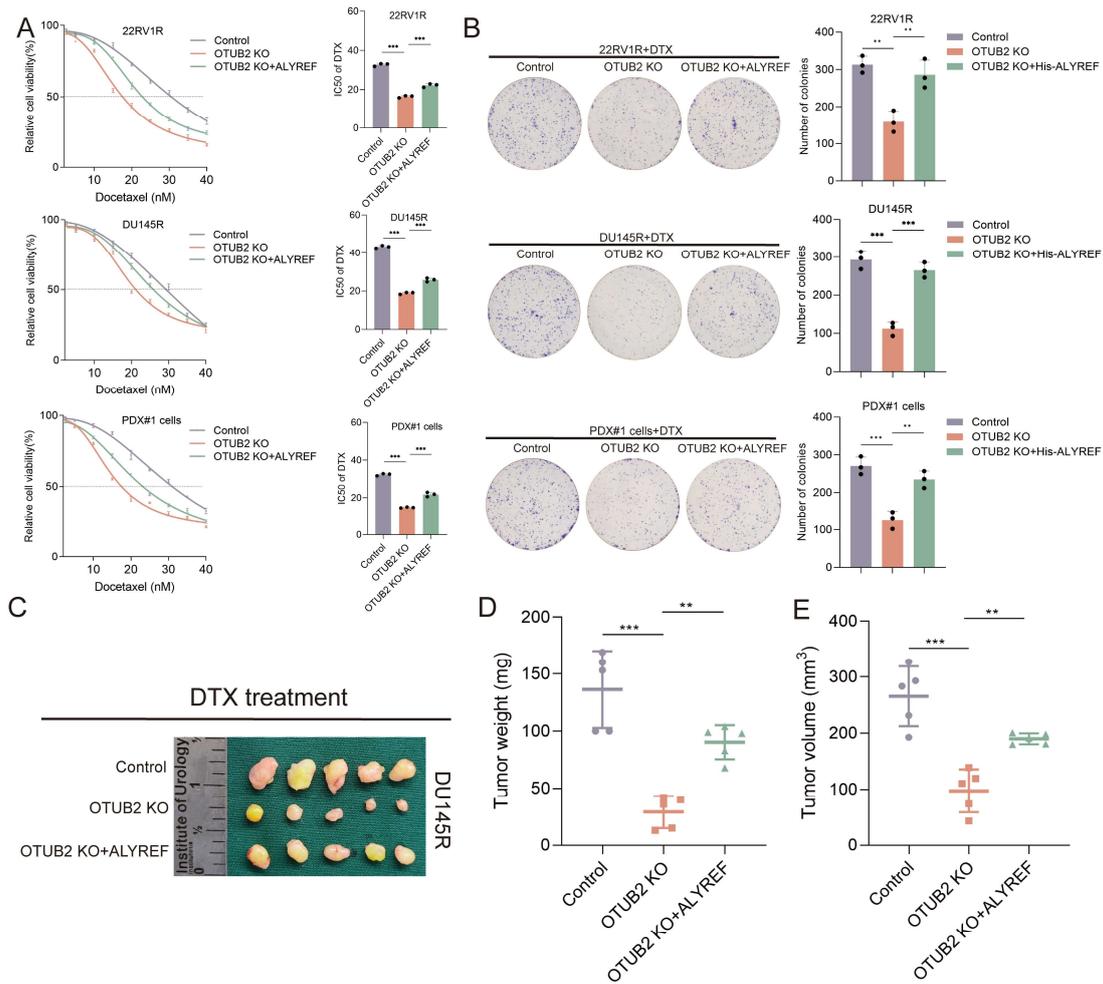
**Figure S1. Construction of DTX-resistant CRPC cells and validation of the role of OTUB2 and YEATS2 on the DTX resistance of CRPC.**

(A-B) Representative images and quantitative results of cloning formation assay showing the difference of DTX-resistant and DTX-sensitive CRPC cells (22RV1R vs. 22RV1; DU145R vs. DU145); three biological replicates were conducted; unpaired two-tailed student's t-test. (C-E) CRPC PDXs from two cases of CRPC patients were transplanted into the flank of male BALB/c nude mice. The lentivirus carrying OTUB2 shRNA was injected into CRPC PDX#1 tumor. Tumor volumes were measured every 3 days. Tumor images, weight and growth curves were obtained after dissection. DTX was injected intraperitoneally every 3 days; two-way repeated measures ANOVA test for D and one-way ANOVA test for E. (F-G) The effect of YEATS2 knockdown on the DTX IC<sub>50</sub> of CRPC cells (22RV1R and DU145R); three biological replicates were conducted; one-way ANOVA test. (H-J) Stable DU145R

cells of indicated groups were injected into the orthotopic prostate of male BALB/c nude mice. DTX was injected intraperitoneally every 3 days from day 12 onwards. Tumor images, weight and volume were obtained after dissection. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

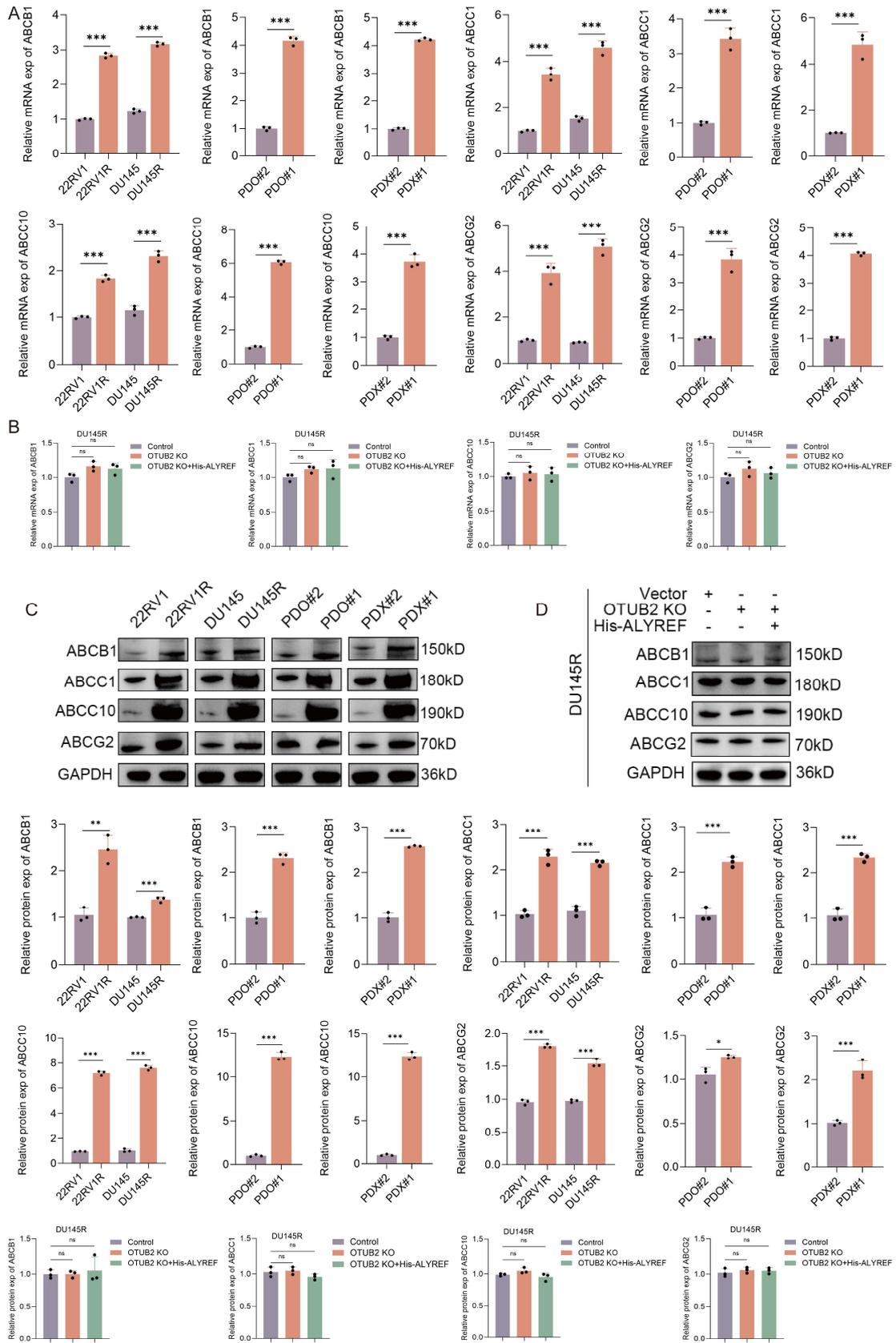


**Figure S2. The secondary diagram of MS of PPM1B (A), SSR3 (B), ERH (C), and CDCC124 (D).**

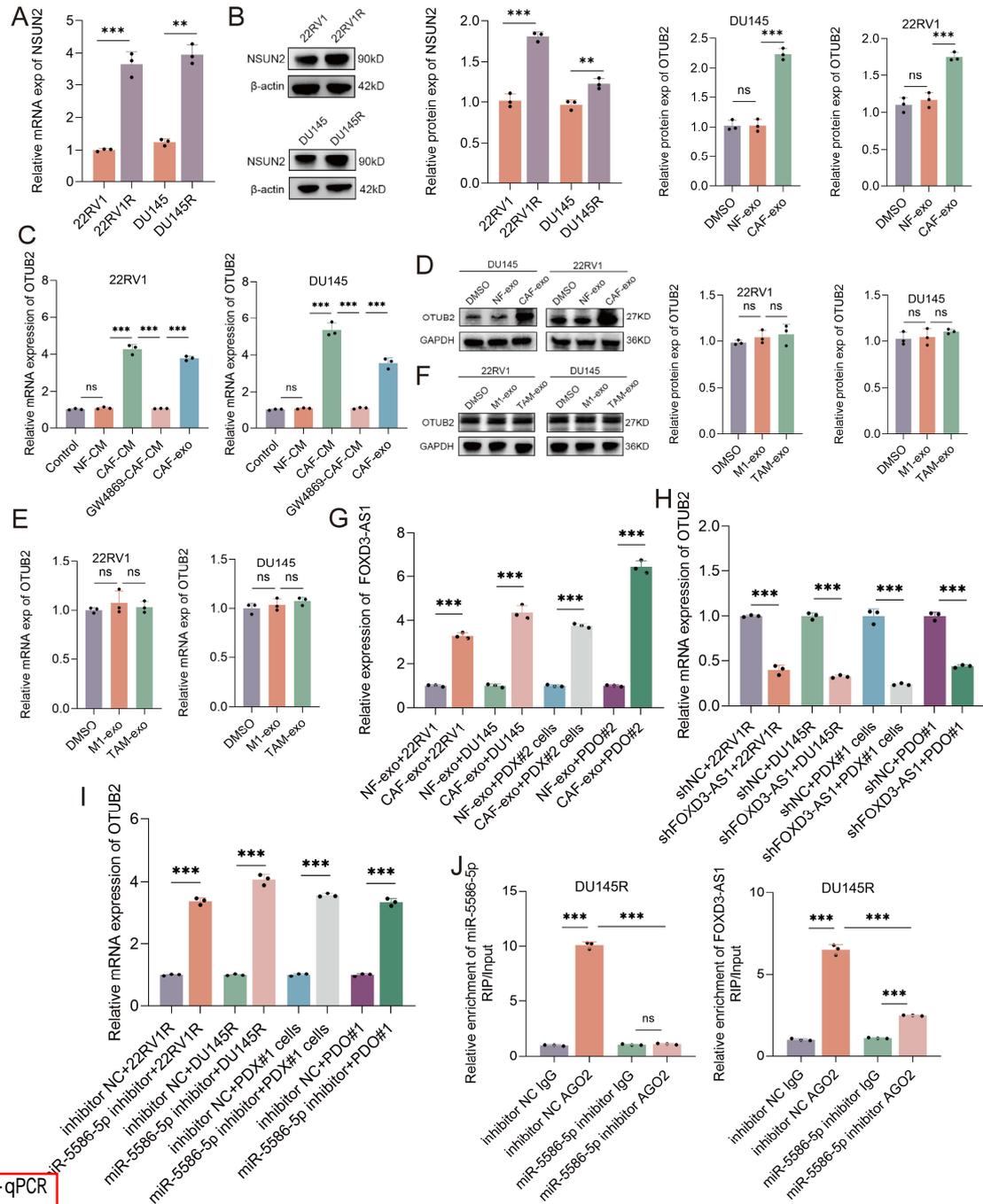


**Figure S3. OTUB2 promoted the DTX resistance of CRPC via upregulating ALYREF *in vivo* and *in vitro*.**

(A) CCK8 toxicity assay showed that the effect of OTUB2 knockout on the IC<sub>50</sub> value of DTX of 22RV1R, DU145R, and PDX#1 cells could be rescued by transducing His-ALYREF; three biological replicates were conducted; one-way ANOVA test. (B) Representative images and quantitative results of cloning formation assay showing the effect of OTUB2 knockout on the cloning formation ability of 22RV1R, DU145R, and PDX#1 cells could be rescued by transducing His-ALYREF; three biological replicates were conducted; one-way ANOVA test. (C-E) Stable DU145R cells of indicated groups were injected into the orthotopic prostate of male BALB/c nude mice. DTX was injected intraperitoneally every 3 days from day 12 onwards. Tumor images, weight and volume were obtained after dissection. One-way ANOVA test. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



ABCC10, and ABCG2 between DTX-resistant and DTX-sensitive CRPC cells (22RV1R vs. 22RV1; DU145R vs. DU145), organoids (PDO#1 vs. PDO#2), and PDXs (PDX#1 vs. PDX#2); three biological replicates were conducted; unpaired two-tailed student's t-test. **(B)** The results of RT-qPCR showing the effect of OTUB2/ALYREF axis on the mRNA expression of ABCB1, ABCC1, ABCC10, and ABCG2 in DTX-resistant CRPC cells; three biological replicates were conducted; one-way ANOVA test. **(C)** The results of western blot showing the difference of the protein expression of ABCB1, ABCC1, ABCC10, and ABCG2 between DTX-resistant and DTX-sensitive CRPC cells (22RV1R vs. 22RV1; DU145R vs. DU145), organoids (PDO#1 vs. PDO#2), and PDXs (PDX#1 vs. PDX#2); three biological replicates were conducted; unpaired two-tailed student's t-test. **(D)** The results of western blot showing the effect of OTUB2/ALYREF axis on the protein expression of ABCB1, ABCC1, ABCC10, and ABCG2 in DTX-resistant CRPC cells; three biological replicates were conducted; one-way ANOVA test. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

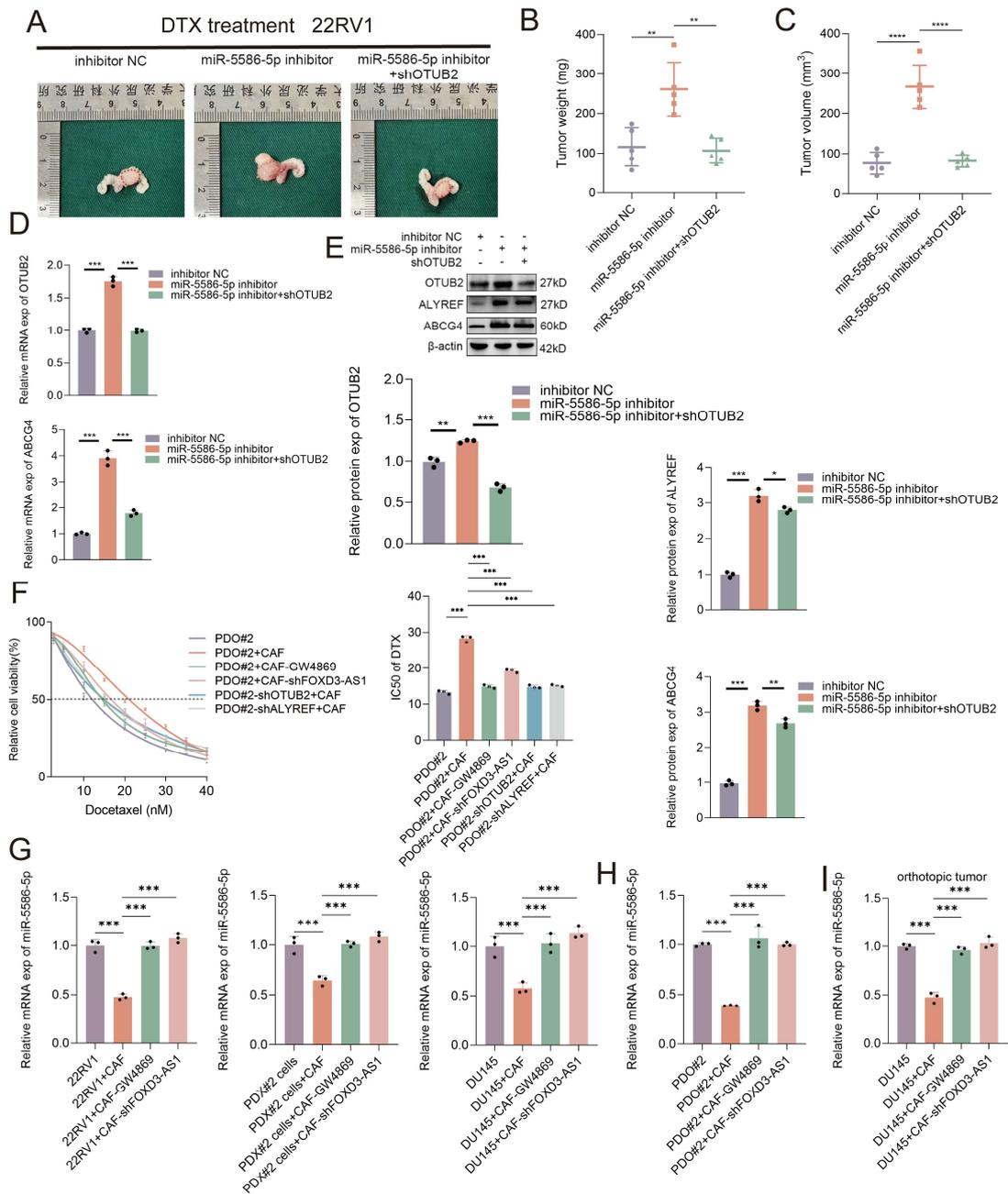


it should be "RT-qPCR and western blot showing the difference of mRNA and protein expression of NSUN2"

**Figure S5. Validation of the differential expression of NSUN2 and CAF-derived exosomal miR-5586-5p upregulating the expression of OTUB2 via sponging FOXD3-AS1**

(A-B) The results of RT-qPCR showing the difference of the mRNA expression of NSUN2 between DTX-resistant and DTX-sensitive CRPC cells (22RV1R vs. 22RV1; DU145R vs. DU145); three biological replicates were conducted; unpaired two-tailed student's t-test. (C) RT-qPCR showing the effect of CAF-derived exosomes on the expression of OTUB2 mRNA in DTX-sensitive CRPC cells (22RV1 and DU145); three biological replicates were conducted; one-way ANOVA test. (D) Western blot showing the effect of CAF-derived exosomes on the expression of OTUB2 protein in DTX-sensitive CRPC cells (22RV1 and DU145); three biological replicates were conducted; one-way ANOVA test. (E) RT-qPCR showing the effect of TAM-derived exosomes on the expression of

OTUB2 mRNA in DTX-sensitive CRPC cells (22RV1 and DU145); three biological replicates were conducted; one-way ANOVA test. **(F)** Western blot showing the effect of TAM-derived exosomes on the expression of OTUB2 protein in DTX-sensitive CRPC cells (22RV1 and DU145); three biological replicates were conducted; one-way ANOVA test. **(G)** RT-qPCR showing the effect of CAF or NFs-derived exosomes treatment on the expression of FOXD3-AS1 in DTX-sensitive CRPC cells (22RV1, DU145, and PDX#2 cells), and organoids (PDO#2); three biological replicates were conducted; unpaired two-tailed student's t-test. **(H)** RT-qPCR showing the effect of FOXD3-AS1 silencing in CRPC cells on the mRNA expression of OTUB2 in DTX-resistant CRPC cells (22RV1R, DU145R, PDX#1 cells), and organoids (PDO#1); three biological replicates were conducted; unpaired two-tailed student's t-test. **(I)** RT-qPCR measuring the relative expression of OTUB2 mRNA in DTX-resistant CRPC cells (22RV1R, DU145R, PDX#1 cells), and organoids (PDO#1) followed by transfection with miR-5586-5p inhibitors; three biological replicates were conducted; unpaired two-tailed student's t-test. **(J)** RIP assays were utilized to measure the endogenous RNA associated with AGO2 in DTX-resistant CRPC cells (DU145R) transfected with miR-5586-5p inhibitor or inhibitor NC; three biological replicates were conducted; one-way ANOVA test. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

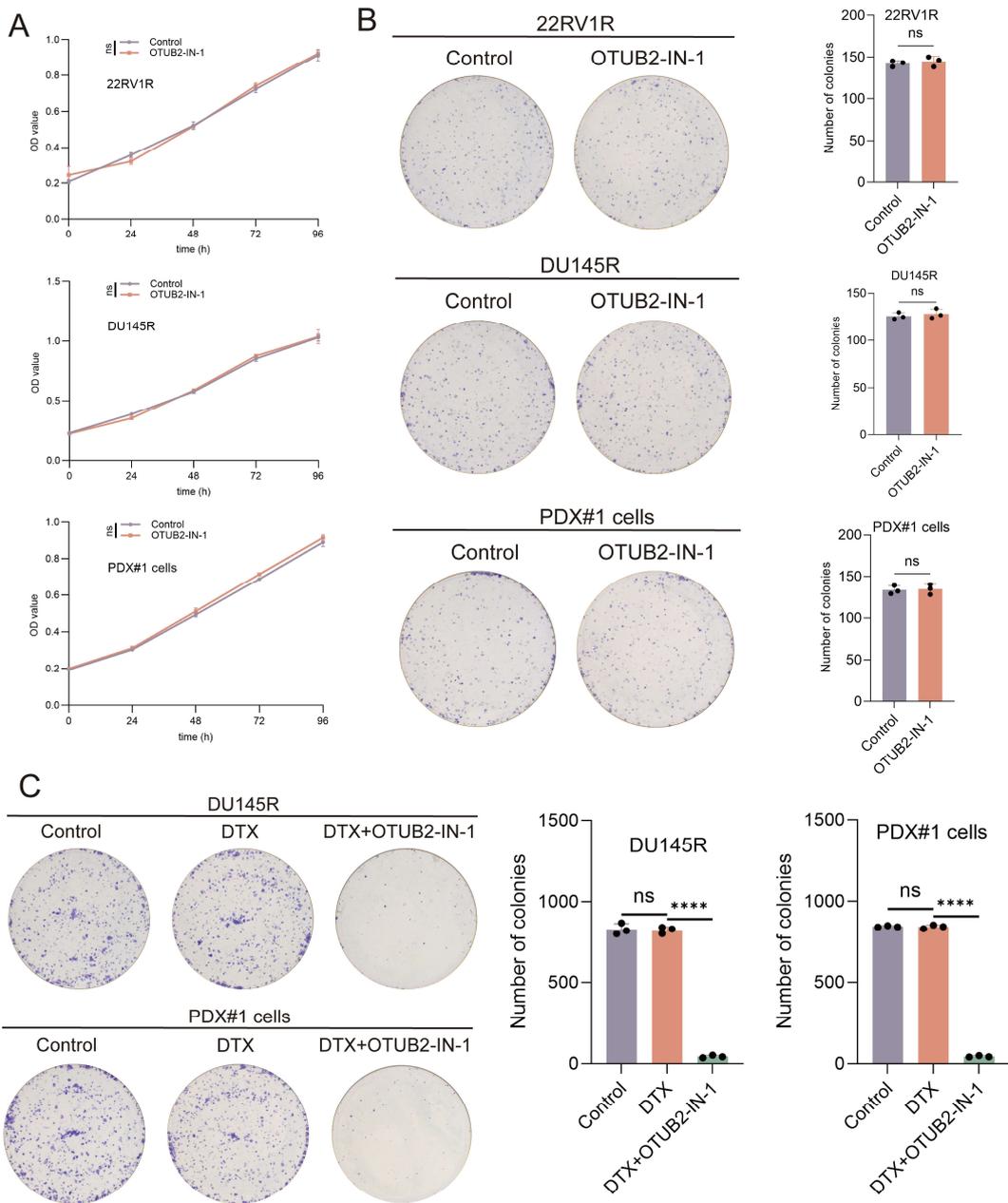


**Figure S6. The role of miR-5586-5p in the DTX resistance and CAF exosomal FOXD3-AS1 facilitates the DTX resistance via regulating OTUB2/ALYREF axis.**

(A-C) 22RV1 cells of indicated group (inhibitor NC, miR-5586-5p inhibitor, miR-5586-5p inhibitor+shOUTB2) were injected into the orthotopic prostate of male BALB/c nude mice. DTX was injected intraperitoneally every 3 days from day 12 onwards. Tumor images, weight and volume were obtained after dissection. One-way ANOVA test. (D) RT-qPCR showing the difference of the expression of OTUB2 mRNA and ABCG4 mRNA in the orthotopic xenografts of indicated group (inhibitor NC, miR-5586-5p inhibitor, miR-5586-5p inhibitor+shOUTB2); three biological replicates were conducted; one-way ANOVA test. (E) Western blot showing the difference of the expression of OTUB2 protein, ALYREF protein, ABCG4 protein in the orthotopic xenografts of indicated group (inhibitor NC, miR-5586-5p inhibitor, miR-5586-5p inhibitor+shOUTB2); three biological replicates

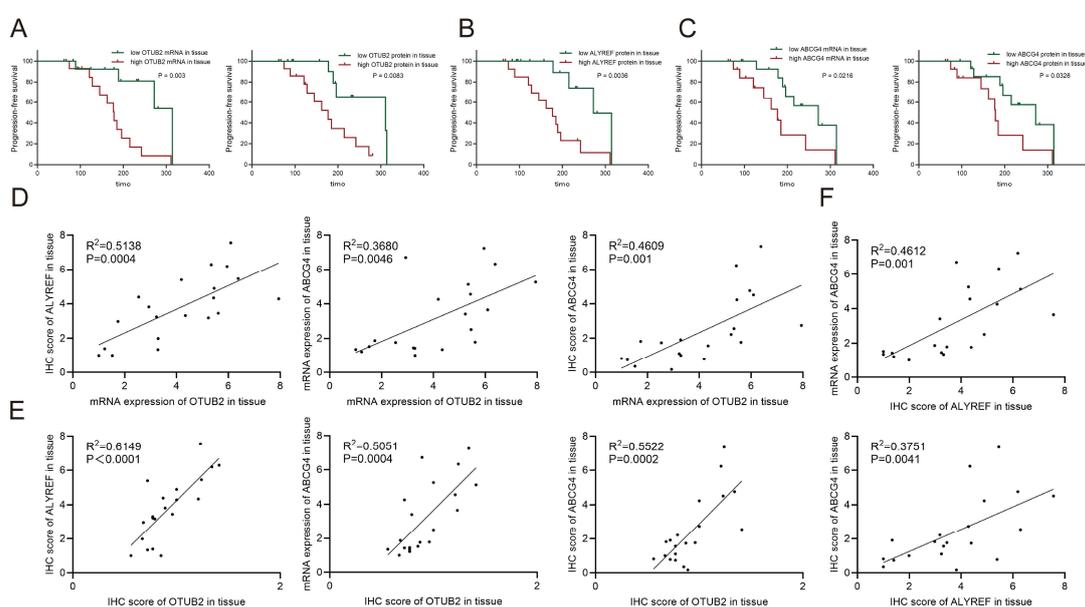
were conducted; one-way ANOVA test. (F) The IC50 value of DTX of DTX-sensitive CRPC organoids (PDO#2) of indicated group. The DTX-sensitive CRPC organoids (PDO#2) were transfected with shOTUB2 or shALYREF. The CAFs were treated with GW4869 or transfected with shFOXD3-AS1. (G) The results of RT-qPCR showing the effect of CAFs-CM on the expression of miR-5586-5p in 22RV1, DU145, PDX#2 cells, and could be rescued by GW4869 treatment in CAFs, or shFOXD3-AS1 in CAFs. (H) The results of RT-qPCR showing the effect of CAFs-CM on the expression of miR-5586-5p in PDO#2, and could be rescued by GW4869 treatment in CAFs, or shFOXD3-AS1 in CAFs. (I) The results of RT-qPCR showing the effect of CAFs on the expression of miR-5586-5p in the orthotopic tumors of indicated group, and could be rescued by GW4869 treatment in CAFs, or shFOXD3-AS1 in CAFs. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

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**Figure S7. Validation of the non-specific toxicity of OTUB2-IN-1 and the effect of combination treatment with OTUB2-IN-1 and DTX.**

(A) CCK8 assay showing the non-specific toxicity of OTUB2-IN-1 on the proliferation ability of DTX-resistant CRPC cells (22RV1, DU145, PDX#1 cells); three biological replicates were conducted; two-way repeated measures ANOVA test. (B) Representative images and quantitative results of cloning formation assay showing the non-specific toxicity of OTUB2-IN-1 on the cloning formation ability of DTX-resistant CRPC cells (22RV1, DU145, PDX#1 cells); three biological replicates were conducted; unpaired two-tailed student's t-test. (C) Representative images and quantitative results of cloning formation assay showing the effect of DTX combining with OTUB2-IN-1 on the cloning formation ability of DU145R and PDX#1 cells; three biological replicates were conducted; one-way ANOVA test. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



**Figure S8. Validation of the OTUB2/ALYREF/ABCG4 axis on the DTX resistance using clinical samples.**

(A-C) The results of Kaplan-Meier survival analysis showing the effect of OTUB2 mRNA, or OTUB2 protein, or ALYREF protein, or ABCG4 mRNA, or ABCG4 protein on the progression-free survival of CRPC patients receiving DTX chemotherapy. (D) The associations of OTUB2 mRNA expression with ALYREF protein (or ABCG4 mRNA, or ABCG4 protein) expression in the tissues of CRPC patients. (E) The associations of OTUB2 protein expression with ALYREF protein (or ABCG4 mRNA, or ABCG4 protein) expression in the tissues of CRPC patients. (F) The associations of ALYREF protein expression with ABCG4 mRNA (or ABCG4 protein) expression in the tissues of CRPC patients. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Table S1. Clinicopathological characteristics of 12 cases of prostate cancer tissue used for PDX construction.**

ID	Pathological type	Age	Gleason score	PSA (ng/mL)	T stage	Castrate resistance	sample source	Metastasis
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PDX#1	Adenocarcinoma	72	8	77.91	T4	Yes	prostate biopsy	bone, lymph node
PDX#2	Adenocarcinoma	78	9	59.72	T4	Yes	prostate biopsy	bone, lymph node
PDX#3	Adenocarcinoma	81	8	17.44	T3	No	radical prostatectomy	No
PDX#4	Adenocarcinoma	67	8	21.73	T3	No	radical prostatectomy	No
PDX#5	Adenocarcinoma	73	9	9.11	T3	No	radical prostatectomy	No
PDX#6	Adenocarcinoma	75	7	17.62	T2	No	radical prostatectomy	No
PDX#7	Adenocarcinoma	65	7	24.78	T4	No	radical prostatectomy	lymph node
PDX#8	Adenocarcinoma	61	9	14.91	T3	No	radical prostatectomy	No
PDX#9	Adenocarcinoma	69	8	18.29	T3	No	radical prostatectomy	No
PDX#10	Adenocarcinoma	77	8	36.27	T3	No	radical prostatectomy	No
PDX#11	Adenocarcinoma	79	10	48.31	T3	No	radical prostatectomy	No
PDX#12	Adenocarcinoma	71	9	55.73	T3	No	radical prostatectomy	No

**Table S2. Clinicopathological characteristics of 30 cases of CRPC patients used for prognosis analysis**

characteristics	Pathological type
Age	68(62-85)
Gleason	
<8	11
≥8	19
PSA	
<20	8
≥20	22
T stage	
T3	12
T4	18
Metastasis	
lymph node	13
bone	25
visceral	7
Disease volume	
low	9
high	21