**Supplementary Table S1.** Key resources**.**

|  |  |  |
| --- | --- | --- |
| **REAGENT or RESOURCE** | **SOURCE** | **IDENTIFER** |
| **Antibodies** |  |  |
| Rabbit anti-AR antibody | Santa Cruz | Cat# sc-816; PRID: AB\_1563391 |
| Goat anti-PSA antibody | Santa Cruz | Cat# sc-7638; PRID: AB\_2134513 |
| Rabbit anti-GFP antibody | Santa Cruz | Cat# sc-8334; PRID: AB\_641123 |
| Rabbit anti-UBE2C antibody | Cell Signaling Technology | Cat# 14234S; AB\_2722751 |
| Mouse anti-GAPDH antibody | Santa Cruz | Cat# sc-47724 HRP; AB\_627678 |
| Rabbit anti-AR antibody | Cell Signaling Technology | Cat# 5153S; AB\_10692774 |
| Rabbit anti- Cleaved Caspase-3 antibody | Cell Signaling Technology | Cat# 9664S; AB\_2070042 |
| Rabbit anti-Ki67 antibody | Abcam | Cat# ab15580; AB\_443209 |
| Mouse anti-goat IgG-HRP antibody | Santa Cruz | Cat# sc-2354; AB\_628490 |
| Goat anti-rabbit IgG-HRP antibody | Santa Cruz | Cat# sc-2004; AB\_631746 |
| Goat anti-mouse IgG-HRP antibody | Santa Cruz | Cat# sc-2005; [AB\_631736](http://antibodyregistry.org/AB_631736) |
| **Chemicals, Peptides and Recombinant**  **Proteins** |  |  |
| Protease inhibitor cocktails | Sigma-Aldrich | Cat# P8340 |
| Fetal Bovine Serum | Atlanta Biologicals | Cat# S11150 |
| Trypsin 0.25% | Corning | Cat# 25-053-CI |
| Penicillin-Streptomycin Solution | Corning | Cat# 30-002-CI |
| L-Glutamine | Corning | Cat# 25-005-CI |
| G418 | Gemini | Cat# 400-111P |
| Puromycin | Sigma-Aldrich | Cat# P8833 |
| RPMI without Phenol Red | Corning | Cat# 17105CV |
| R1881 | PerkinElmer | Cat# NLP005005MG |
| E2 | Sigma-Aldrich | Cat# E2758 |
| Dexamethasone | Sigma-Aldrich | Cat# D4902 |
| Enzalutamide | Medchem Express | Cat# HY-70002 |
| Matrigel | Corning | Cat# 354234 |
| PolyJet™ In Vitro DNA Transfection  Reagent | SignaGen Laboratories | Cat# SL100688 |
| RNase A | Thermo Fisher Scientific | Cat# EN0531 |
| proteinase K | NEW ENGLAND Biolabs | Cat# P8107S |

**Supplementary Table S1.** Key resources, cont.

|  |  |  |
| --- | --- | --- |
| **REAGENT or RESOURCE** | **SOURCE** | **IDENTIFER** |
| **Chemicals, Peptides and Recombinant**  **Proteins cont.** |  |  |
| salmon sperm DNA/Protein A agarose | Millipore | Cat# 16-157 |
| PrimeScript RT Reagent Kit | TaKaRa | Cat# RR037A |
| SYBR Advantage qPCR Premix | TaKaRa | Cat# 639676 |
| SYBR Green PCR mix | Thermo Fisher Scientific | Cat# 4309155 |
| RNAlater-ICE | Thermo Fisher Scientific | Cat# AM7030 |
| Recombinant human androgen receptor | Sigma-Aldrich | Cat# SRP2161 |
| Antigen unmasking solution | Vector Laboratories | Cat# H-3300 |
| Critical Commercial Assays |  |  |
| RNeasy Mini Kit | Qiagen | Cat# 74106 |
| Western ECL Substrate | BioRad | Cat# 170506 |
| BCA Protein Assay Kit | ThermoFisher | Cat# 23225 |
| Dual-Luciferase Reporter Assay kit | Promega | Cat# E1980 |
| Human Kallikrein 3/PSA Quantikine  ELISA Kit | R&D systems | Cat# DKK300 |
| QIAquick PCR purification kit | Qiagen | Cat# 28106 |
| ImmunoCruz rabbit ABC Staining System | Santa Cruz | Cat# sc-2018 |
| NovaRED Peroxidase (HRP) Substrate Kit | Vector Laboratories | Cat# SK-4800 |
| Deposited Data |  |  |
| RNA-seq | This study | GSE115395 |
| **Experimental Models: Cell Lines** |  |  |
| Human: LNCaP | ATCC | CRL-1740 |
| Human: 22Rv1 | ATCC | CRL-2505 |
| Human: PC3 | ATCC | CRL-1435 |
| Human: VCaP | ATCC | CRL-2876 |
| Human: C4-2 | 41 | N/A |
| Human: LAPC4 | 42 | N/A |
| Human: CWR-R1 | 43 | N/A |
| Human: GFP-ARv7:PC3 | 26 | N/A |
| Human: PC3-AR | 44 | N/A |

**Supplementary Table S1.** Key resources, cont.

|  |  |  |
| --- | --- | --- |
| **REAGENT or RESOURCE** | **SOURCE** | **IDENTIFER** |
| **Experimental Models:**  **Organisms/Strains** |  |  |
| Crl:SHO-PrkdcscidHrhr mice | Charles River | 474 |
| Oligonucleotides |  |  |
| qRT-PCR primers, see Table S1 | This study | N/A |
| qRT-PCR primers (ChIP assay), see Table  S1 | 19 | N/A |
| **Recombinant DNA** |  |  |
| pEGFP-C1 | Clontech | Cat# 632470 |
| pEGFP-C1-AR | 27 | N/A |
| pEGFP-C1-NAR | 27 | N/A |
| pEGFP-C1-ER | 48 | N/A |
| pRL-TK | Promega | Cat# E2241 |
| pPSA6.1-Luc | 49 | N/A |
| MMTV-Luc | 31 | N/A |
| 3x ERE TATA-Luc | Addgene | Cat# 11354 |
| pEGFP-C1-ARv7 | Addgene | Cat# 86856 |
| **Software and Algorithms** |  |  |
| Image-Pro Plus 6.0 | Media Cybernetics | <http://www.mediacy.com/imagepropl>  us |
| GraphPad Prism 6.0 | GraphPad Software | https://[www.graphpad.com/scientific-](http://www.graphpad.com/scientific-)  software/prism/ |
| Cutadapt v1.8.3 | 50 | <http://cutadapt.readthedocs.io/en/stabl>  e/guide.html |
| HISAT2 v2.1.0 | 51 | https://ccb.jhu.edu/software/hisat2/in  dex.shtml |
| HTSeq v0.9.0 | 52 | https://htseq.readthedocs.io/en/release  \_0.9.1/ |
| R Bioconductor package (edgeR) | 53 | https://bioconductor.org/packages/rel  ease/bioc/html/edgeR.html |
| R | R | https[://w](http://www.r-project.org/)ww.[r-project.org/](http://www.r-project.org/) |

**Supplementary Table S2**. List of oligonucleotides used in the study

|  |  |
| --- | --- |
| **qRT-PCR Primers:** | **Sequence** |
| GAPDH-F | AGTCCACTGGCGTCTTCAC |
| GAPDH-R | TCTTGAGGCTGTTGTCATACTTC |
| EAF2-F | TGAGGTTGGTGAAGGTGAACA |
| EAF2-R | AGCCGACATTCTCCAGTATCAT |
| NKX3-1-F | GGAGAGGAAGTTCAGCCATCA |
| NKX3-1-R | TAGTCTTATAGCGTCTGTTCTGGA |
| AR V7-F | CCATCTTGTCGTCTTCGGAAATGTTATGAAGC |
| AR V7-R | TTTGAATGAGGCAAGTCAGCCTTTCT |
| AR1-F | CGCCAAGGAGTTGTGTAAGG |
| AR1-R | CTGTCGTCTAGCAGAGAACCT |
| PSA-F | AGGCCTTCCCTGTACACCAA |
| PSA-R | GTCTTGGCCTGGTCATTTCC |
| FKBP5\_F | CCGCAGGATATACGCCAACA |
| FKBP5\_R | TTCTCTTCTTCCATTGCTTGACTG |
| UBE2C\_F | TGGAACAGTATATGAAGACCTGAG |
| UBE2C\_R | TGGATGGAGAGCAGAATGGT |
| CCNA2\_F | CAAGAGGACCAGGAGAATATCAAC |
| CCNA2\_R | CCAAGGAGGAACGGTGACAT |
| BUB1B\_F | CTCTGGCTTCTCTGGTTCTTCT |
| BUB1B\_R | ACTTAGGCATTGGTCTGTCTTCT |
| KIF15\_F | AGGAAGAACTGACCAAGAAGGA |
| KIF15\_R | CAGCAGCAGAATCCATCACAT |
| **ChIP qPCR Primers:** | **Sequence** |
| PSA-EN-ARE-F | TGGGACAACTTGCAAACCTG |
| PSA-EN-ARE-R | CCAGAGTAGGTCTGTTTTCAATCCA |
| PSA-PR-ARE-F | CCTAGATGAAGTCTCCATGAGCTACA |
| PSA-PR-ARE-R | GGGAGGGAGAGCTAGCACTTG |
| TMPRSS2-ARE-F | TGGTCCTGGATGATAAAAAAAGTTT |
| TMPRSS2-ARE-R | GACATACGCCCCACAACAGA |

**Supplementary Table S3.** Pairwise comparisons of enzalutamide vs Compounds

|  |  |  |  |
| --- | --- | --- | --- |
| **Filter** | **IMTPPE** | **(+)-JJ-450** | **(-)-JJ-450** |
| Adjusted P-value ** 0.05** | 10202 | 10187 | 459 |
| Fold change ** 2** | 1187 | 1321 | 27 |
| Fold change ** -2** | 1683 | 1573 | 25 |
| Adjusted P-value ** 0.05**  & absolute fold change ** 2** | 2856 | 2878 | 36 |

**Supplementary Table S4.** Pairwise comparisons of control vs enzalutamide/(-)-JJ-450

|  |  |  |
| --- | --- | --- |
| **Filter** | **(-)-JJ-450** | **enzalutamide** |
| Adjusted P-value ** 0.05** | 1635 | 4831 |
| Fold change ** 2** | 67 | 176 |
| Fold change ** -2** | 24 | 43 |
| Adjusted P-value ** 0.05**  & absolute fold change ** 2** | 56 | 186 |