Supplementary Methods

qPCR primer sequences

For AR target gene assessment, primers were as follows: GAPDH fwd: 5'-GAAGGTGAAGGTCGGAGTC-3', GAPDH rev: 5' GAAGATGGTGATGGGATTTC-3', PSA fwd: 5'-AGGCCTTCCCTGTACACCAA-3', PSA rev: 5'-GTCTTGGCCTGGTCATTTCC-3', TMPRSS2 fwd: 5'- CTGCCAAGGTGCTTCTCATT-3', TMPRSS2 rev: 5'-CTGTCACCCTGGCAAGAATC-3', FKBP5 fwd: 5'- AGGAGGGAAGAGTCCCAGTG-3', FKBP5 rev: 5'- TGGGAAGCTACTGGTTTTGC-3', hK2 fwd: 5'-CTGTCAGAGCCTGCCAAGAT-3', hK2 rev: 5'- GCAAGAACTCCTCTGGTTCG-3'.

For splicing factor knockdown and assessment of AR-V7 and total AR levels, the following primers were used: GAPDH fwd and rev (see above), AR exon 1 fwd: 5'-TGGATGGATAGCTACTCC GG-3', AR exon 2 rev: 5'-CCCAGAAGCTTCATCTCCAC-3', AR exon 3 fwd: 5'-AACAGAAGTACCTGTGCGCC-3', AR CE3 rev: 5'-TCAGGGTCTGGTCATTTT GA-3', SRSF1 fwd: 5'-GAAACTGCCTCAATCCGGGT-3', SRSF1 rev: 5'-GTAACTGCGACTCCTGCTGT-3', U2AF2 fwd: 5'-CAGGCCTCACGACTACCAG-3', REV: 5'-CAGGCCTCACGACTACCAG-3', PTBP1 fwd: 5'-CGGGGATCTGACGAGCTTTT-3', PTBP1 rev: 5'-TCGGCTGTCACCTTTGAACT-3'.

For AR-V7 RNAi, CPSF complex RNAi, and morpholino experiments, the following primers to detect AR-Vs and full length AR were used: AR exon 3 fwd: 5'-AAC AGA AGT ACC TGT GCG CC-3', CE1 rev: 5'-TGA GAC TCC AAA CAC CCT CA-3' (AR-V1), CE2 rev: 5'-TAT GAC ACT CTG CTG CCT GC-3' (AR-V6), CE5: 5'-GCAAATGTCTCCAAAAAGCAGC-3' (AR-V9). AR-

V7 was detected with AR exon 3 fwd2: 5'-CGGAAATGTTATGAAGCAGGGATGACTC-3', CE3 rev: 5'-CCAGACTATCCACTAGAGCCCTCT-3'. Primers used in this study to detect full length AR included AR Ex7 fwd: 5'-TACCAGCTCACCAAGCTCCTG-3', AR Ex8 rev: 5'-GAAAGTCCACGCTCACCATGTG-3'.

For CPSF complex siRNA screening, the following primers were used: CPSF1 fwd: 5'-GTGTACAAACAGGCGCATCC-3', CPSF1 rev: 5'-TCATTCTTGGTCAGAGCCTCG-3', CPSF2 fwd: 5'-GAGGCCTGACGAGATTAATAAAGA-3', CPSF2 rev: 5'-AGGGCAGATTCTTCTTGGACC-3', CPSF3 fwd: 5'-GGAGTGACGGAAGTTGTGCT-3', CPSF3 rev: 5'-AGCTCCAAGGGGTCGGATCA-3', CPSF4 fwd: 5'-CCCTCGATTTGAACTGCCCA-3', CPSF4 rev: 5'-TCCTTTCTCGCCACACTTGTA-3', WDR33 fwd: 5'-GAGCGGGATTGAGAGGATCG-3', WDR33 rev: 5'-TAGGATACGTCTGGCTTTGAGC-3', FIP1L1 fwd: 5'-GGAGCACCACAGTATGGGAG-3', FIP1L1 rev: 5'-ATCAGCACCAGGTTTACGCC-3'.

CPSF complex knockdown experiments used 18S primers as an internal control as described in (1). The sequences were as follows: 18S fwd: 5'-CAGCCACCCGAGATTGAGCA-3', 18S rev: 5'-TAGTAGCGACGGGCGGTGTG-3'. AR-V7 siRNA and morpholino experiments used Actin2 fwd: 5'-ATGCAGAAAGAGATCACCGC-3', Actin2 rev: 5'-ACATCTGCTGGAAGGTGGAC-3' as internal controls.

AR RNA Sequencing

Sequencing libraries were pooled and diluted to 10 pM for flow cell clustering and sequenced using Illumina HiSeq 2000 with 2X50bp settings. Illumina reads were aligned to hg19 using TopHat (v.2.0.11) (2) with --no-coverage-search and --micro-exon-search enabled, and the mean and

standard deviation for the inner distance between mate pairs set empirically. AR and AR-V isoforms have been annotated (3). Annotated AR and AR-V isoforms from the UCSC annotation database were used as a guide for TopHat alignment. Aligned reads in .bam format were visualized in IGV. The TopHat junctions.bed output file was parsed to determine how many reads supported novel and canonical splice junctions within the AR locus.

TCGA RNA-seq

Raw RNA-seq data from the TCGA was downloaded from dbGaP, study accession phs000178.v9.v8 (4) , yielding paired tumor/normal samples for 52 patients. Genes under 300 bp were removed from further analysis as these are not isolated effectively in standard RNA-seq library preps. Genes with low expression (those with less than 10 reads in half of the samples) were removed, and paired tumor and normal samples were analyzed for differential expression using edgeR. In edgeR, the ROAST gene set test was used to test whether known splicing genes were differentially expressed in the cancer samples (5).

SU2C RNA-seq

Raw RNA-seq reads from metastatic biopsies were obtained from dbGaP, study accession phs000554.v1.p1. For analysis of AR RNA-seq data, Illumina reads were aligned to hg19 using TopHat (v.2.0.11) with --no-coverage-search and --micro-exon-search enabled, and the mean and standard deviation for the inner distance between mate pairs set empirically (2). Annotated AR and AR-V isoforms from the UCSC annotation database were used as a guide for TopHat alignment. Aligned reads in .bam format were visualized in integrative genomics viewer (IGV) (6). The TopHat junctions.bed output file was parsed to determine how many reads supported novel and canonical splice junctions within the AR locus.

AR subcellular fractionation

22Rv1-undup3 cells were seeded in RPMI medium supplemented with 10% CSS for 24 hours and treated with either ethanol (vehicle) or 1 nM mibolerone (synthetic androgen) for 24 hours. Cells were washed with 1X PBS, harvested in hypotonic buffer, and subjected to subcellular fractionation as described in (7). Lysates in 1X Laemmli buffer were loaded in equal volumes on either 7.5% or 4-15% polyacrylamide gels (Biorad TGX) and electrophoresed at 200v for 30 minutes. Western blots were performed as described (8). Blots were incubated with antibodies to AR (Santa Cruz Santa Cruz Biotechnology N-20, 1:2000), alpha-tubulin (Cell Signaling Technologies DM1A, 1:1000), and Histone-H3 (Abcam di methyl K4 [Y47], 1:5000).

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