Supplementary Methods

LC-MS/MS Sample Preparation: Stock mixtures of stable isotope-labeled standards were prepared in either methanol or acetonitrile at a concentration of 10 mg/mL and then diluted to lower concentrations. Tumor tissues were flash frozen in liquid nitrogen collected and stored at -80^{o}C until use. Aliquots of tumor tissue (~50-250 mg) were weighed and placed in Eppendorf microcentrifuge tubes. Samples were kept on ice throughout the processing steps. Each sample was spiked with 10 µL of 9 androgen internal standard mixtures at a concentration of 5 ng/mL for T (2,3,4-$^{13}C_3$), Prog (2,3,4-$^{13}C_3$), AED (2,3,4-$^{13}C_3$) and DOC (2,2,4,6,6,17α,21,21-D_8), 50 ng/mL DHT (2,3,4-$^{13}C_3$), F (9,11,12,12-D_4) and A5-diol (16,16,17-D_3), and 500 ng/ml for DHEA (2,2,3,4,4,6-D_6) and Preg (20,21-$^{13}C_2$; 16,16-D_2). After an hour, each sample was re-suspended in PBS, then 0.9-2.0 mm blend stainless steel beads (Next Advance, Troy, NY) were added. The volume ratio of tissue, PBS, and beads is 1:2:1. Samples were homogenized at speed 8,000 rpm for 5 minutes at 4^{o}C (Bullet Blender, Next Advance, Troy, NY) and transferred to a 5 mL glass centrifuge tube for Liquid-Liquid Extraction (LLE) (1) followed by Solid Phase Extraction (SPE) (2). Briefly, 2 mL of MTBE was added to the glass centrifuge tube with sample homogenate in it and then vortexed for 30 seconds. All samples were centrifuged at 2,000 x g for 10 minutes at 5^{o}C to separate the organic and aqueous layers. The top organic layer was decanted, and the remaining androgens from the aqueous phase were extracted two more times. The pooled MTBE androgen extract was dried under nitrogen (5 psi) using Reacti-Vap™ Evaporators (TS 18826, Thermo Fisher Scientific, Waltham, MA) in a fume hood at room temperature. The androgen pellet was re-dissolved in 2 mL of aqueous 20% methanol and loaded into Isolute C18 cartridges (200 mg/3 mL), which were conditioned with 5 mL methanol and followed by 3 mL water. After loading the sample and washing the cartridges with 2 mL of aqueous 10% methanol, the androgens were eluted with 2 mL 100% methanol. This eluate was dried and reconstituted with 50 µL or 100 µL of aqueous 50% methanol depending on the amount of tissue for each sample. The concentrated extract was transferred to an LC-MS autosampler vial (Fisher Scientific, Hampton, NH) and injected right away.

RNA sequencing: Mice bearing orthotopic prostate tumors were cardiac perfused with PBS (without heparin), following by resection of tumors and immediate freezing in liquid nitrogen. RNA from prostate tumor tissue was extracted using RNeasy® Plus Mini kit (Qiagen) according to manufacturer's protocol with on-column DNase digestion and screened for quality on an Agilent Tape-Station (Agilent Technologies, Inc., Wilmington DE). RNA-sequencing libraries were prepared using the NuGen Ovation Mouse RNA-Seq Kit System (Tecan US, Inc., Morrisville, NC). Briefly, 100 ng of DNase treated RNA was used to generate cDNA and a strand-specific library following the manufacturer's protocol. Library molecules containing ribosomal RNA sequences were depleted using the NuGen Mouse AnyDeplete probe-based enzymatic process. The final libraries were assessed for quality on the Agilent

TapeStation (Agilent Technologies, Inc., Wilmington DE), and quantitative RT-PCR for library quantification was performed using the Kapa Library Quantification Kit (Roche Sequencing, Pleasanton, CA). The libraries were sequenced on the Illumina NextSeq 500 v2 sequencer with a 75-base paired-end run in order to generate about 40 million read pairs per sample. Data are available via Gene Expression Omnibus (GSE180270).

RNA-seq analysis: The raw reads were first assessed for quality using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and then trimmed by cutadapt (3) (version 1.8.1) to remove reads with adaptor contaminants and low-quality bases. Read pairs with either end too short (<25bps) were discarded from further analysis. Trimmed and filtered reads were aligned to the GRCm38 mouse reference genome using STAR (4) (version 2.5.3a). Unmapped reads were further aligned to GRCh37 human reference genome. Uniquely mapped reads were counted by htseq-count (5) (version 0.6.1) using Gencode m21 annotation for mouse genome and Gencode v30 annotation for human gene ERG. Differential expression analysis was performed using DESeq2 taking into account of RNA composition bias (6). Genes with fold-change>2 and false discovery rate (FDR) q-values< 0.05 were considered differentially expressed. For each differential expression analysis comparing treated groups vs. IgG group, mouse genes were converted to their human homologs and ranked based on –log10(p-value)*(sign of log2(fold-change)). The ranked gene list used to perform pre-ranked gene set enrichment analysis (GSEA(7) version 4.0.2) to assess enrichment of hallmarks, curated gene sets, and gene ontology (8) terms in MSigDB (7). The resulting normalized enrichment score (NES) and FDR controlled p-values were used to assess the transcriptome changes induced by Lupron and α-CSF1.

Supplementary References

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