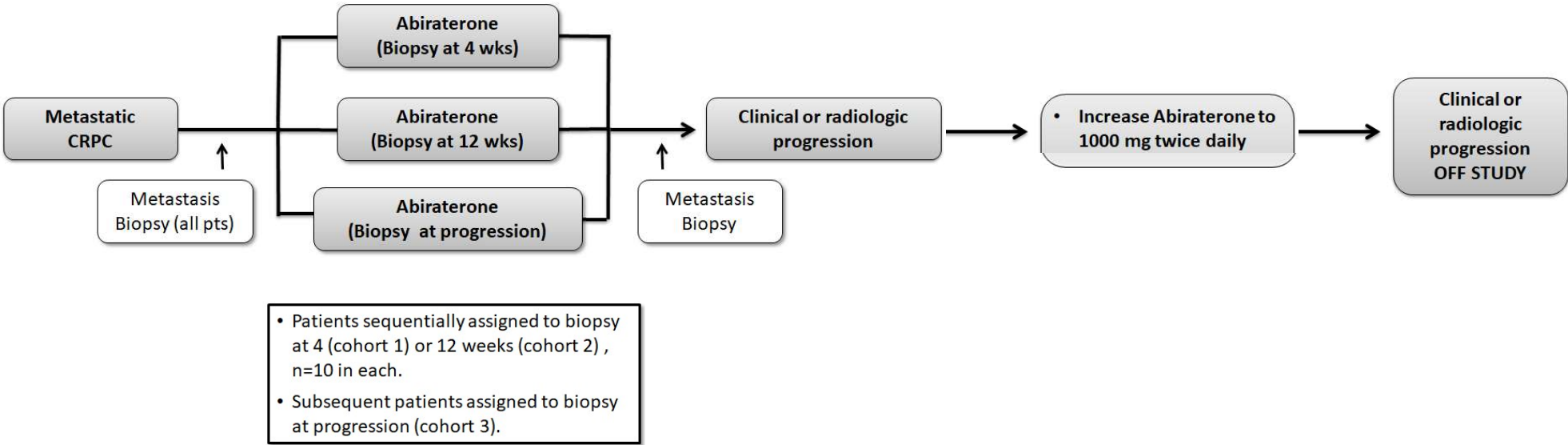
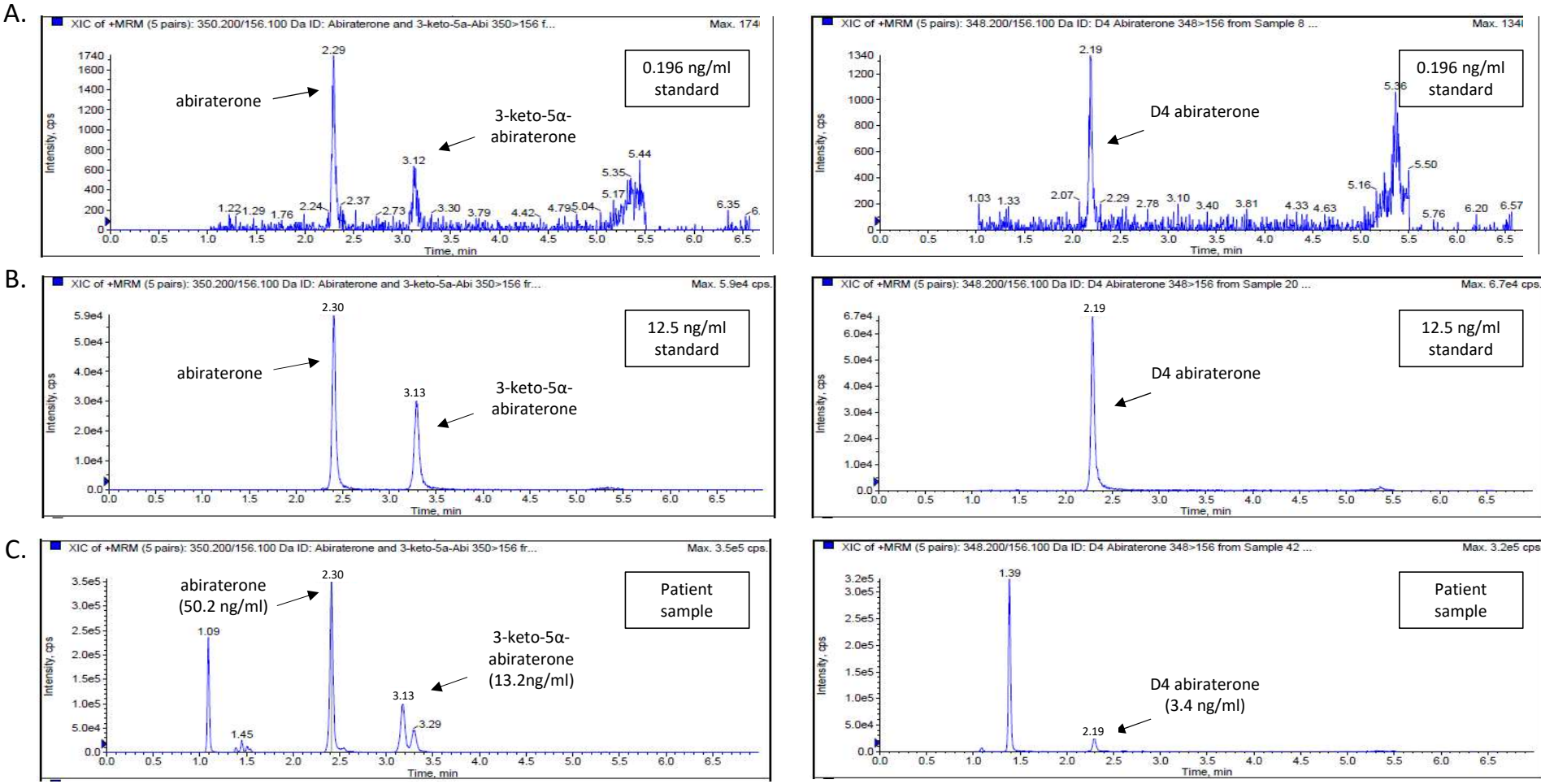


Supplementary Figure 1. Study Schema



Supplemental Figure 1. Study Schema and Sample Collection and Processing. Blood was drawn at baseline, and at 4, 8, and 12 weeks and at progression for assessment of steroid and drug levels. In patients who underwent escalation of AA to 2000mg, blood was also drawn at 4 weeks after dose increase and at time of second progression. Blood was separately processed to obtain buffy coat and serum by standard methods and all samples were frozen at -80 degrees. Core biopsies of tumor metastases were obtained at baseline in all patients, after 4 weeks of therapy (cohort 1), 12 weeks (cohort 2), or at progression (cohort 3). In all cases except one, the second biopsy was performed from the same metastatic site that was sampled at baseline. Tissue cores were immediately snap frozen in liquid nitrogen and maintained at -80 degrees. All serum, buffy coat and tissue samples were batched and then thawed and assayed for androgens or sequencing after completion of the study.

Supplementary Figure 2. Additional Mass Spectrometry Methods



Supplementary Figure 2. Additional Mass Spectrometry Methods. Chromatograms showing the peak and retention times for the Abiraterone, D4 abiraterone and 3-keto-5 α -abiraterone standards at **A.** 0.196 ng/ml and **B.** 12.5ng/ml. Analytes were detected using multiple reaction monitoring (MRM), at a characteristic ion dissociation transition of m/z 350.00 \rightarrow 156 for abiraterone and 3-keto-5 α -abiraterone, and 348.00 \rightarrow 155 for D4 abiraterone. **C.** Representative patient sample with chromatograms for intermediate concentrations of each analyte.

Lower limits of detection and quantitation (LLOD and LLOQ) for steroids in serum were 0.002 ng/ml for AED, testosterone; 0.005 ng/ml for progesterone, pregnenolone, androsterone, DHEA; 0.01 ng/ml for dihydrotestosterone (DHT); and 0.6 ng/ml for DHEAS. The LLOD and LLOQ for steroids in tissue were 0.49 pg/sample (0.01 pg/mg) for AED, testosterone 0.98 pg/sample (0.02 pg/mg) for progesterone, androsterone; 1.96 pg/sample (0.04 pg/mg) for pregnenolone; 3.9 pg/sample (0.08 pg/mg) for DHT; 31.2 pg/sample (0.8 pg/mg) for DHEA; and 98 pg/sample (2.0 pg/mg) for DHEAS. For DHEAS only, a low standard curve was used for the post-treatment tissue samples with LLOD and LLOQ set at 0.38 pg/sample (0.01 pg/mg). Of note, while true peaks were visible for the reported data points, the accuracy of the measurements is uncertain as the recovery of DHEAS (based on the internal standard) was <10% in this assay. The LLOD and LLOQ for abiraterone, D4-abiraterone, and 3-keto-5 α -abiraterone was 0.20 ng/ml in serum and 0.078 ng/sample (1.6 pg/mg) in tissue.

Supplementary Table 1. Serum and Tissue Steroid Levels prior to Abiraterone Acetate and Prednisone Therapy

Serum at baseline	PSA (ng/dl)	Preg (ng/ml)	Prog (ng/ml)	DHEAS (ng/ml)	DHEA (ng/ml)	AED (ng/ml)	ASD (ng/ml)	T (ng/ml)	DHT (pg/ml)
Mean	142	0.287	0.023	646	1.14	0.358	0.0461	0.069	0.017
Median	78.0	0.263	0.019	502	0.793	0.307	0.046	0.067	0.013
Minimum	2.0	0.037	0.005	36	0.083	0.019	0.005	0.006	0.010
Maximum	908.0	0.894	0.068	2659	3.510	0.954	0.128	0.187	0.052
Lower 95% CI of median	30.0	0.161	0.014	230	0.460	0.234	0.024	0.047	0.010
Upper 95% CI of median	162.0	0.357	0.022	895	1.650	0.439	0.066	0.089	0.019

Tissue at first biopsy (pg/mg)	Preg	Prog	DHEAS	AED	ASD	T	DHT*
Mean	17.6	0.234	71.3	0.148	0.053	0.167	0.17
Median	12.7	0.10	47.8	0.117	0.035	0.055	0.08
Minimum	4.42	0.02	2.0	0.01	0.019	0.01	0.08
Maximum	78.1	2.19	340	0.534	0.215	1.16	0.88
Lower 95% CI of median	9.64	0.065	18.7	0.071	0.02	0.03	
Upper 95% CI of median	19.1	0.224	88.4	0.234	0.049	0.175	

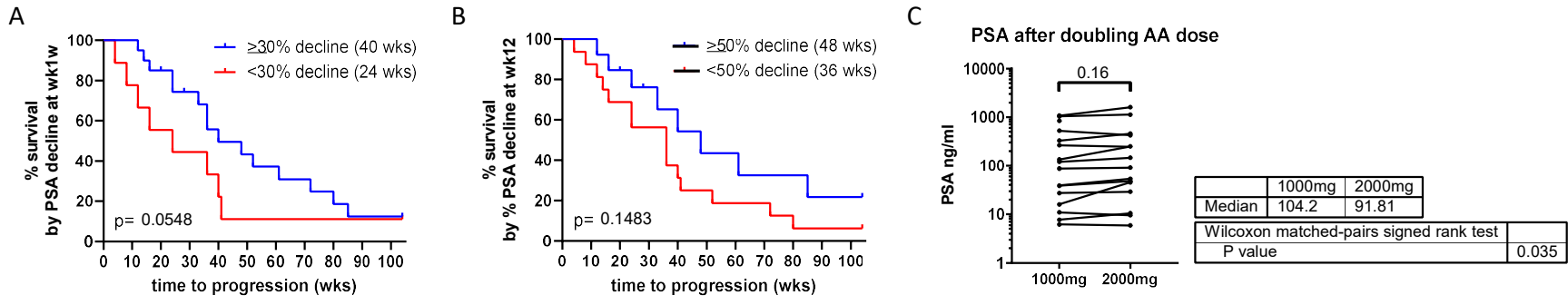
*DHT was below the limit of quantitation (0.08 pg/mg) in 25 of 29 samples

Supplementary Table 2. Serum and Tissue Steroid Levels on Abiraterone Acetate and Prednisone Therapy

Serum (ng/ml)		d0	wk4	wk8	wk12	EOS
Mean	Preg	0.287	2.27	2.77	2.36	1.97
Median	Preg	0.263	0.719	0.97	1.56	0.90
Minimum	Preg	0.037	0.142	0.112	0.30	0.113
Maximum	Preg	0.894	11.70	25.42	7.97	9.08
Lower 95% CI of median	Preg	0.161	0.461	0.421	0.886	0.534
Upper 95% CI of median	Preg	0.357	4.17	2.275	2.775	1.605
Mean	DHEAS	646	3.52	3.66	2.84	2.68
Median	DHEAS	502	1.80	1.20	2.40	0.80
Minimum	DHEAS	36	0.60	0.60	0.60	0.60
Maximum	DHEAS	2659	15.6	20.8	11.6	20.0
Lower 95% CI of median	DHEAS	230	0.90	0.80	0.90	0.60
Upper 95% CI of median	DHEAS	895	4.60	3.00	3.70	2.40
Mean	DHEA	1.14	0.009	0.0136	0.013	0.015
Median	DHEA	0.793	0.007	0.008	0.009	0.005
Minimum	DHEA	0.083	0.005	0.005	0.005	0.005
Maximum	DHEA	3.510	0.031	0.038	0.052	0.179
Lower 95% CI of median	DHEA	0.460	0.005	0.005	0.005	0.005
Upper 95% CI of median	DHEA	1.650	0.010	0.017	0.017	0.009
Mean	AED	0.358	0.003	0.003	0.005	0.005
Median	AED	0.307	0.002	0.002	0.002	0.002
Minimum	AED	0.019	0.002	0.002	0.002	0.002
Maximum	AED	0.954	0.008	0.013	0.029	0.031
Lower 95% CI of median	AED	0.234	0.002	0.002	0.002	0.002
Upper 95% CI of median	AED	0.439	0.003	0.003	0.003	0.003
Mean	Testosterone	0.0691	0.0024	0.0026	0.0028	0.0028
Median	Testosterone	0.0672	0.0024	0.0024	0.0024	0.0024
Minimum	Testosterone	0.0064	0.0024	0.0024	0.0024	0.0024
Maximum	Testosterone	0.1873	0.0024	0.0054	0.0060	0.0075
Lower 95% CI of median	Testosterone	0.0466	0.0024	0.0024	0.0024	0.0024
Upper 95% CI of median	Testosterone	0.0898	0.0024	0.0024	0.0024	0.0024

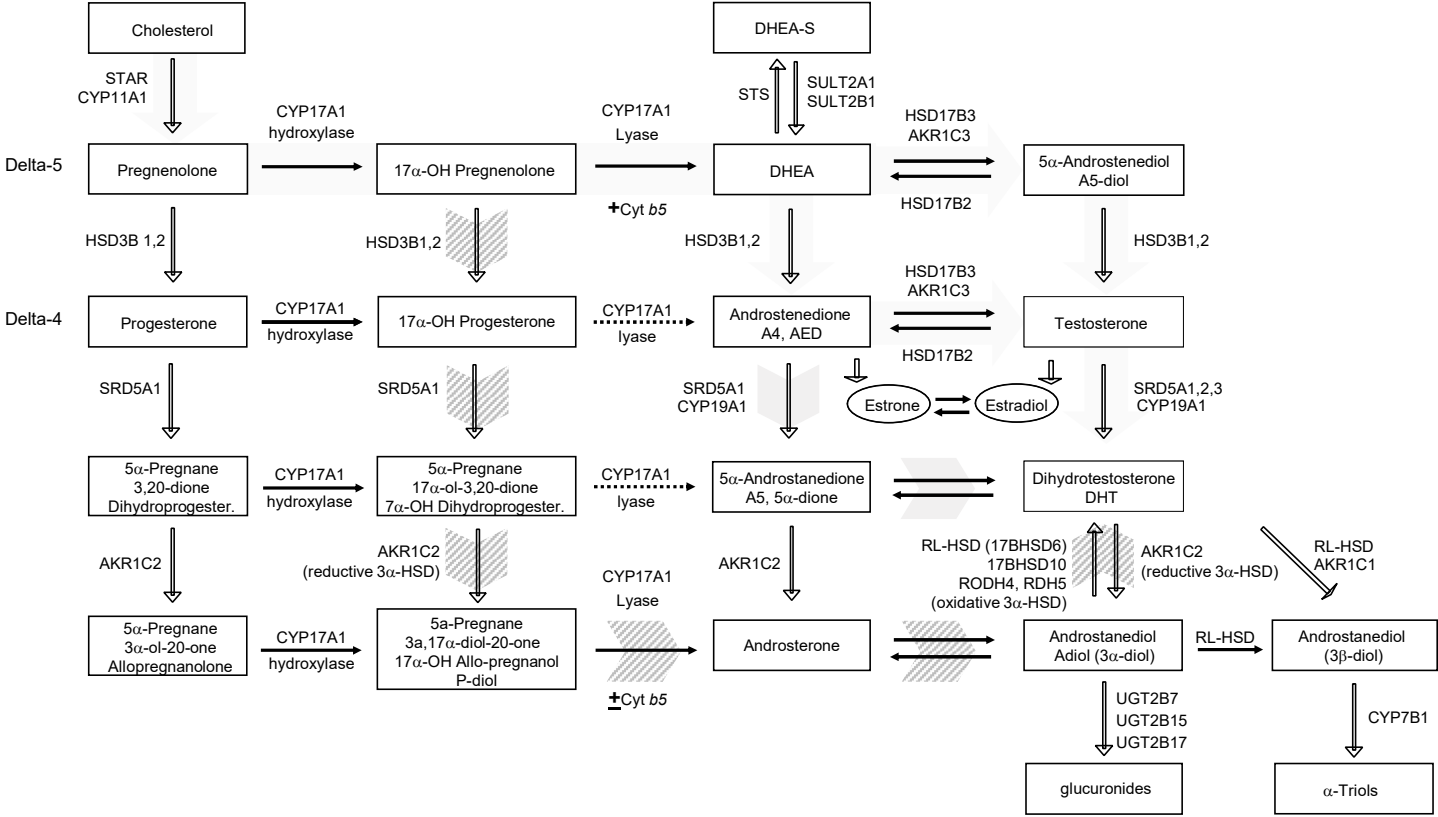
Tissue Biopsies (pg/mg)		bx1	bx2	4wk bx	12wk bx	EOS bx
Mean	Preg	17.6	39.6	27	32.6	59.8
Median	Preg	12.7	22.9	26.2	14.2	34.1
Minimum	Preg	4.42	4.56	4.56	6.68	8.56
Maximum	Preg	78.2	176	71.7	102	176
Lower 95% CI of median	Preg	9.64	11.7	9.44	6.68	8.56
Upper 95% CI of median	Preg	19.1	46.6	37.8	102	176
Mean	DHEAS	71.2	0.83	0.61	0.55	1.27
Median	DHEAS	47.8	0.37	0.55	0.49	0.01
Minimum	DHEAS	2.0	0.01	0.01	0.01	0.01
Maximum	DHEAS	340	9.04	1.52	1.56	9.04
Lower 95% CI of median	DHEAS	18.70	0.01	0.01	0.01	0.01
Upper 95% CI of median	DHEAS	88.40	0.81	1.38	1.56	1.42
Mean	AED	0.148	0.010	0.010	0.010	0.014
Median	AED	0.117	0.010	0.010	0.010	0.010
Minimum	AED	0.010	0.010	0.010	0.010	0.010
Maximum	AED	0.534	0.039	0.010	0.010	0.039
Lower 95% CI of median	AED	0.071	0.010	0.010	0.010	0.010
Upper 95% CI of median	AED	0.234	0.010	0.010	0.010	0.039
Mean	Testost.	0.156	0.011	0.011	0.011	0.011
Median	Testost.	0.046	0.010	0.010	0.010	0.010
Minimum	Testost.	0.010	0.010	0.010	0.010	0.010
Maximum	Testost.	1.15	0.019	0.017	0.015	0.019
Lower 95% CI of median	Testost.	0.012	0.010	0.010	0.010	0.010
Upper 95% CI of median	Testost.	0.168	0.010	0.010	0.015	0.019

Supplementary Figure 3. Survival by PSA Decline



Supplementary Figure 3. Kaplan Meier plot of time to radiographic progression on standard dose abiraterone acetate (AA) as a function of achieving **A.** 30% PSA decline or **B.** 50% PSA decline at 12 weeks. **C.** PSA change one month after doubling dose of AA to 2000mg daily.

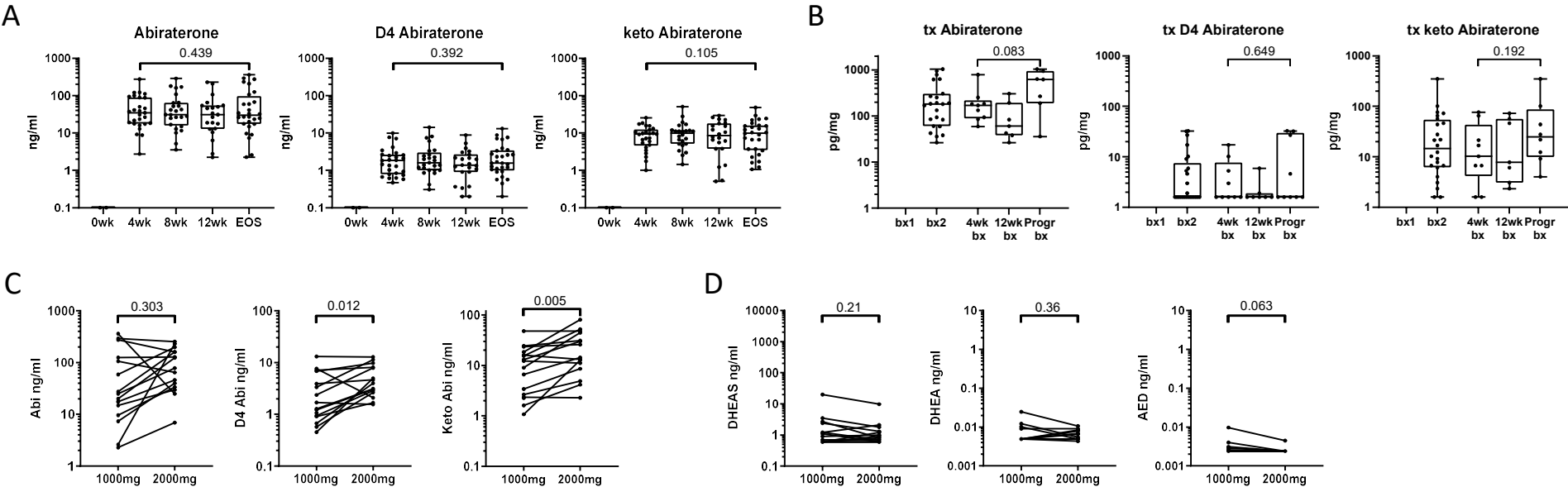
Supplementary Figure 4. The Steroid Metabolic Pathway



Supplementary Table 3. Levels of Abiraterone and Metabolites in Serum and Tissue

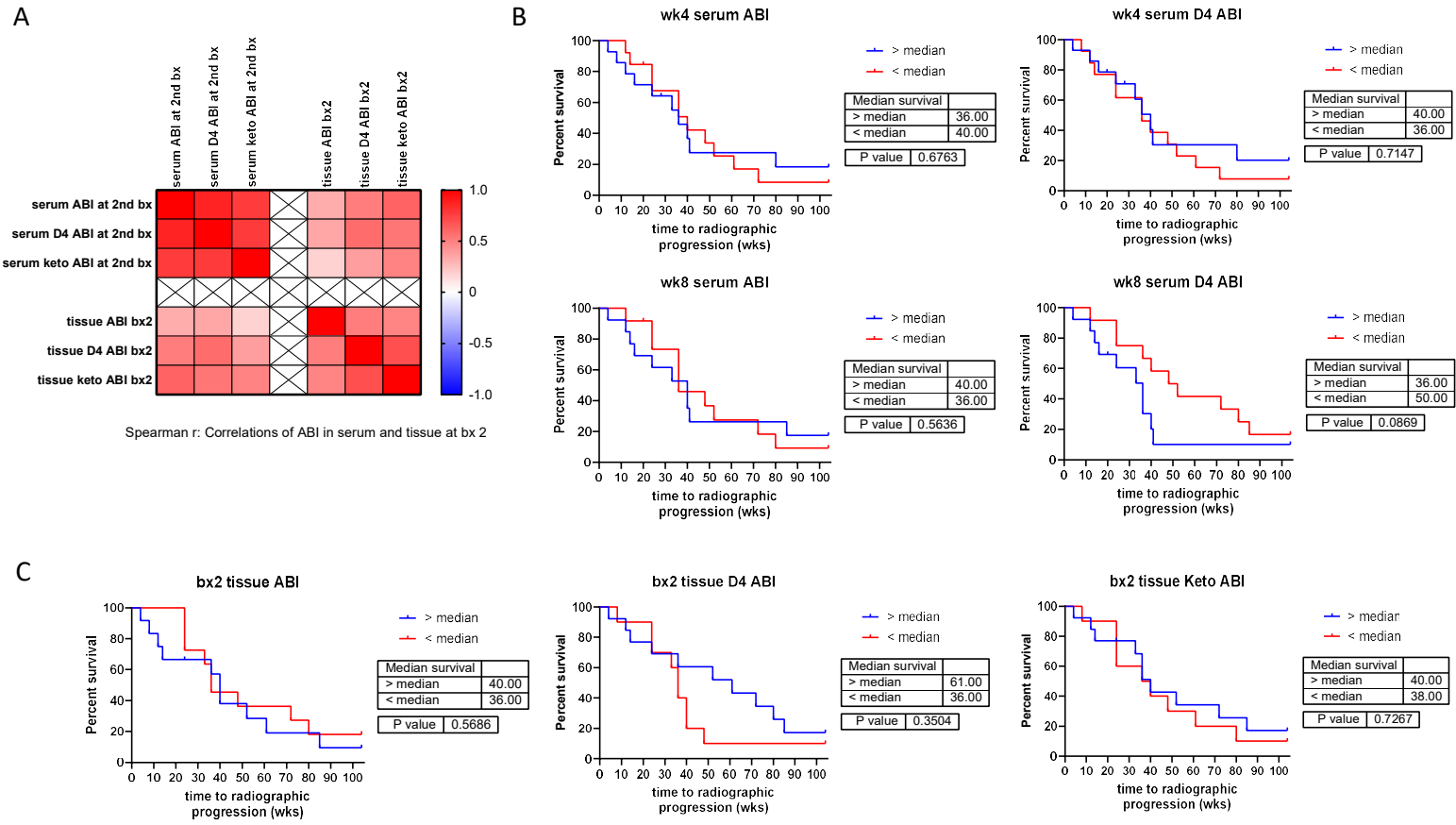
Abiraterone and Metabolites		4wk	8wk	12wk	EOS		4wk	8wk	12wk	EOS
						Serum (ng/ml)				
Mean	Abi	54.5	60.4	50.2	77		279	216	107	533
Median	Abi	34.8	31.1	30.9	29.8		182	170	60.4	622
Minimum	Abi	2.75	3.57	2.25	2.27		26.6	59.2	26.6	35.9
Maximum	Abi	275	288	228	361		1036	789	305	1036
Lower 95% CI of median	Abi	18.4	24.8	13.2	20.1		83.7	84.9	26.6	35.9
Lower 95% CI of median	Abi	88	53	54.2	59.8		267	252	305	1036
						2nd Tissue biopsy (pg/mg)				
Mean	D4 Abi	2.4	2.8	2.0	2.9		6.9	4.9	2.3	11.6
Median	D4 Abi	1.8	1.6	1.4	1.6		1.6	1.6	1.6	1.6
Minimum	D4 Abi	0.5	0.3	0.2	0.2		1.6	1.6	1.6	1.6
Maximum	D4 Abi	10.0	14.2	8.8	13.1		32.7	17.2	5.9	32.7
Lower 95% CI of median	D4 Abi	0.8	1.2	0.9	1.1		1.6	1.6	1.6	1.6
Lower 95% CI of median	D4 Abi	2.6	2.5	2.2	2.8		5.3	10.2	5.9	32.1
						Serum (ng/ml)				
Mean	Keto-Abi	9.2	11.4	11.2	11.9		40.5	23.0	27.9	71.3
Median	Keto-Abi	9.4	10.0	8.6	10.0		14.6	10.2	7.8	24.8
Minimum	Keto-Abi	1.0	1.5	0.5	1.1		1.6	1.6	2.4	4.0
Maximum	Keto-Abi	25.6	50.8	29.5	48.0		349.4	76.4	72.8	349.4
Lower 95% CI of median	Keto-Abi	5.4	5.2	3.8	3.7		6.7	1.6	2.4	4.0
Lower 95% CI of median	Keto-Abi	10.6	11.7	17.8	15.4		46.5	65.1	72.8	349.4

Supplementary Figure 5. Serum and Tissue Levels of Abiraterone and Metabolites



Supplementary Figure 5. Serum and Tissue Levels of Abiraterone and Metabolites. Change in levels of abiraterone, D4 abiraterone and 5 α -keto abiraterone in **A**. serum after standard dose abiraterone acetate at 4, 8 and 12 weeks (4wk, 8wk, 12wk) and at end of study (EOS) at the time of radiographic progression. **B**. Change in abiraterone and metabolite levels in metastatic tissue (tx) biopsies while on therapy (biopsy 2 – bx2). Biopsy 2 was taken at either 4 weeks (4wk bx), 12 weeks (12wk bx), or at progression (Progr bx). Dehydroepiandrosterone sulfate (DHEAS), dehydroepiandrosterone (DHEA), androstenedione (AED). Before and after plots of **C**. Abiraterone and metabolite levels, and **D**. serum DHEAS levels before and after escalation from 1000mg to 2000mg per day. P values for the indicated comparison calculated via paired two sample t tests. Data are shown as box and-whisker plots, where horizontal lines indicate median values; white boxes denote the 75th (upper margin) and 25th percentiles (lower margin), and upper and lower bars indicate the minimum and maximum values, respectively.

Supplementary Figure 6. Associations of Serum and Tissue Abiraterone and Metabolite Levels



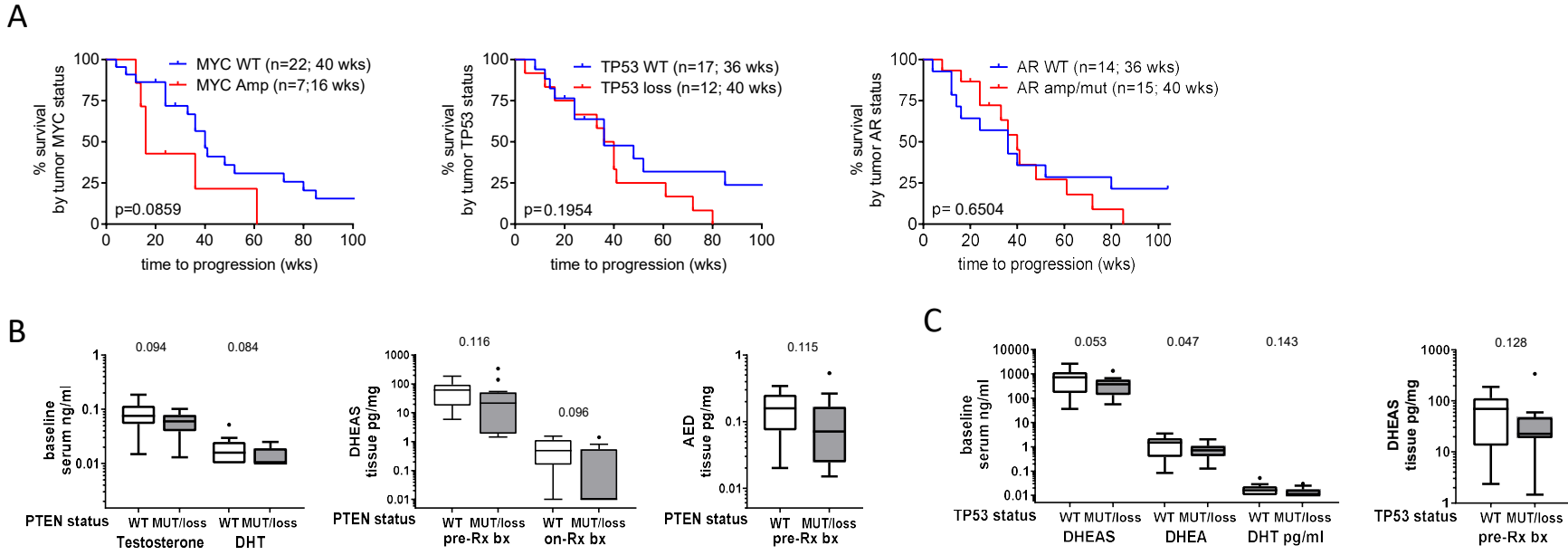
Supplementary Figure 6. Associations of Serum and Tissue Abiraterone (ABI) and Metabolite Levels. **A.** Heatmap of Spearman correlations in serum and tissue at time of second tissue biopsy (bx 2). **B.** Radiographic progression free survival (rPFS) as a function of serum ABI and D4-abiraterone levels (D4 ABI) at week 4 (wk4) and week 8 (wk8) comparing subjects above vs below the median. **C.** rPFS as a function of tissue abiraterone (ABI), D4 ABI, and Keto ABI levels at time of second biopsy (wk4 or wk12). Progression-free survival was estimated using Kaplan-Meier methods and compared using the Gehan-Wilcoxon test.

Supplementary Table 4. Tumor Genomic Alterations

patient ID	Estimated % tumor content	PTEN	MYC	TP53	RB1 copy loss	BRCA2	PIK3	AR mutation/amp	TMPRSS2-ERG	other tumor genetic change	HSD3B1 genotype
7639-05	4%										HTZ
7639-07	4%										HTZ
7639-09	4%										WT
7639-22	4%									ATM M/?LOH	WT
7639-16	4%			M/LOH						NOTCH1 HC CG; NF1 CL	WT
7639-33	4%	BACL		M/LOH	CL	CL		A		AKT A	WT
7639-10	5%	BA ex2 del			CL*			M		AR p.T878A; APC M; KDM6M; CHD1 CL*	HTZ
7639-23	10%	CL					M	A	F	PIK3CA M	WT
7639-11	15%	M/LOH		M/LOH			CG			PIK3CA/CB copy gain; PLK2 focal hom loss	WT
7639-31	35%						M	M	F	AR p.W742L; PIK3CA M; MSI/MLH1 & hypermut; FOXA1 rearrang	HTZ
7639-01	40%	M		M/?LOH			M	A	F	PIK3CA activating M; APC M, FLT4 M	MT
7639-15	45%	BACL		M/?LOH				M	F	AR p.W742C; EZH2 gain	WT
7639-13	50%								F	CCND1 A; FOXA1 A; KDM6A M PLZF hom CL	MT
7639-18	55%			M/LOH				A	F	BRIP1 rearrangement; KDM6A ex 3-6 del	HTZ
7639-03	60%	BACL						M	F	APC M/LOH; AR p.T878A; ETV5 F	HTZ
7639-04	60%	BACL		M/LOH	CL*			A		BRIP1 rearrangement	WT
7639-08	60%		A					A		ATM M/LOH/BA; MDM4 A; CCND1 A	HTZ
7639-20	60%		A	M/LOH		M/LOH				BRAF CG	WT
7639-19	65%	BA ex1-7		M/LOH	CL	CL				TET CG	MT
7639-27	65%				CL			M		AR p.H875Y; SPOP p.F133L M; CHDA M/LOH/BA; FOXA1 M	MT
7639-30	65%		A			BACL				SPOP p.F133V M, PLK2 & EPHA5 BACL	HTZ
7639-06	70%	BACL		BACL				A	F	NF1 exon 1-35 del	WT
7639-32	70%									ARID1A M	WT
7639-26	75%		A	M/LOH				A		SPOP p.F133V; CHD1 BACL	HTZ
7639-02	80%		A	BA ex1-4del		BACL					MT
7639-21	80%			CL						CDK12 M&TDS; CCND1 A; MDM4 A;	WT
7639-25	80%	BA ex2 del	A					A		CDK12 M&TDS; CCND1 A	WT
7639-28	80%	BACL		M/LOH			M	ex5-7 del	F	PIK3R1 M; GRIN2A M	MT
7639-29	85%	BACL	A	M/LOH	CL	CL					HTZ

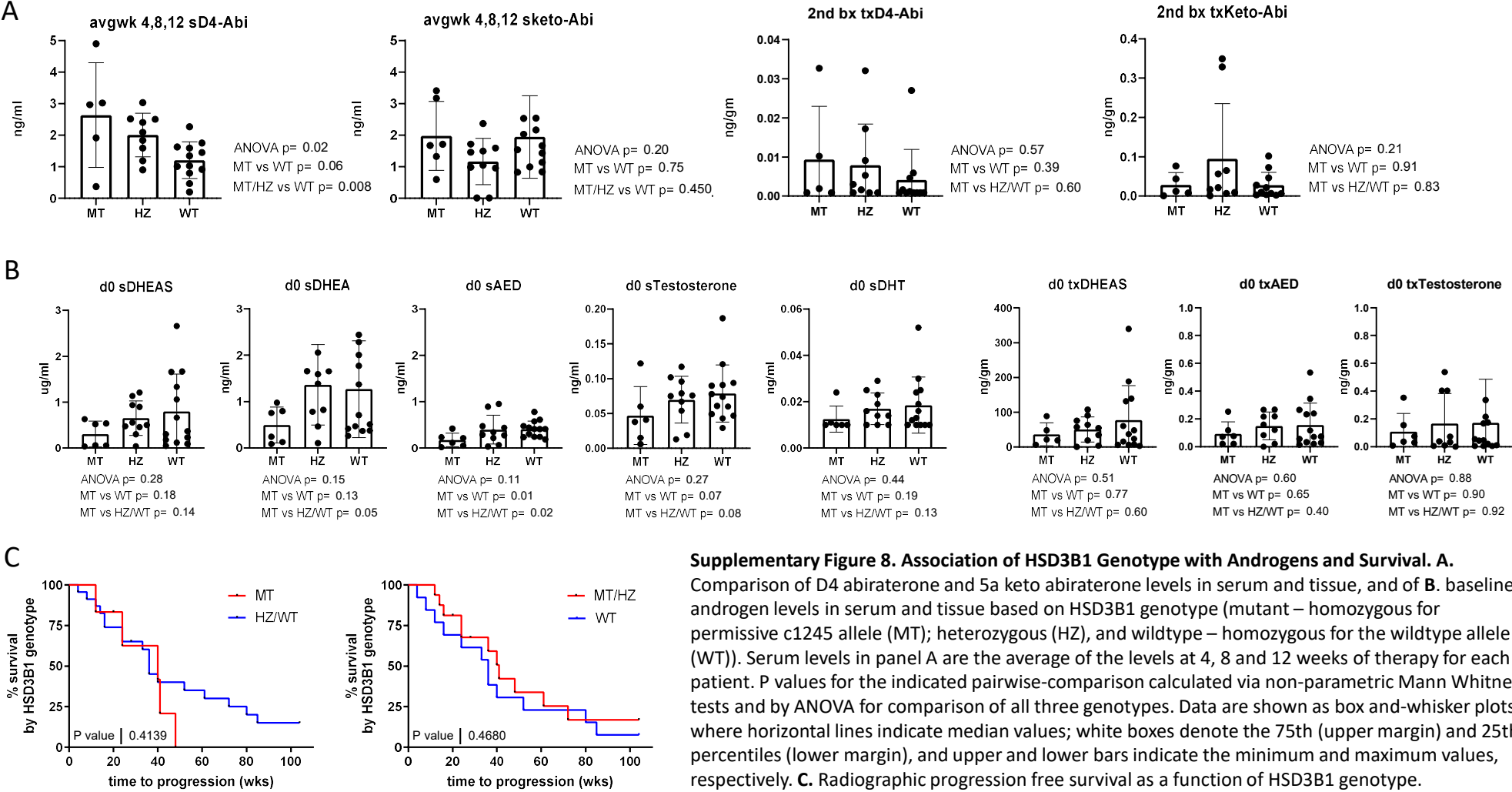
A - Amplification; BACL - biallelic copy loss; CL* - single vs double loss ambiguous; CG - copy gain; F - fusion; M - mutation; LOH - loss of heterozygosity
 WT - wild type; MT - mutant; HTZ - heterozygous

Supplementary Figure 7. Association of Tumor Genomic Alterations with Survival and Androgen Levels



Supplementary Figure 7. Association of Tumor Genomic Alterations with Survival and Androgen Levels. **A.** Radiographic progression free survival (rPFS) as a function of MYC, TP53 or AR status. **B.** Comparison of pre-treatment levels of testosterone and DHT in serum, and of DHEAS and AED in metastatic tissue biopsies based on tumor PTEN status. **C.** Comparison of pre-treatment levels of DHEAS, DHEA and DHT in serum, and of DHEAS in metastatic tissue biopsies based on tumor TP53 status.

Supplementary Figure 8. Association of HSD3B1 Genotype with Androgens and Survival



Supplementary Figure 8. Association of HSD3B1 Genotype with Androgens and Survival. A.

Comparison of D4 abiraterone and 5a keto abiraterone levels in serum and tissue, and of **B.** baseline androgen levels in serum and tissue based on HSD3B1 genotype (mutant – homozygous for permissive c1245 allele (MT); heterozygous (HZ), and wildtype – homozygous for the wildtype allele (WT)). Serum levels in panel A are the average of the levels at 4, 8 and 12 weeks of therapy for each patient. P values for the indicated pairwise-comparison calculated via non-parametric Mann Whitney tests and by ANOVA for comparison of all three genotypes. Data are shown as box and-whisker plots, where horizontal lines indicate median values; white boxes denote the 75th (upper margin) and 25th percentiles (lower margin), and upper and lower bars indicate the minimum and maximum values, respectively. **C.** Radiographic progression free survival as a function of HSD3B1 genotype.