

SUPPLEMENTAL DATA

Supplemental Table 1

	Overall	Pairwise comparison		
	<i>P</i> -value	WT vs <i>KCNJ5</i>	<i>KCNJ5</i> vs <i>ATPIA1</i>	<i>ATPIA1</i> vs <i>CACNA1D</i>
CYP11B1	0.002	0.004	0.201	1.000
CYP11B2	0.005	1.000	<0.001	0.021
CYP17A1	0.428	NA	NA	NA
HSD3B1	0.551	NA	NA	NA
HSD3B2	0.356	NA	NA	NA

Relationships between staining intensities of steroidogenic enzymes and genotypes.

Staining intensities of three cores from each patient were classified into highest, median and lowest intensity. Median intensity of three cores from each patient were used for immunohistochemistry profiles of steroidogenic enzymes. *P*-values of less than 0.05 were considered significant. NA = not applicable. Enzymes with higher staining intensity were shown as bolds.

Supplemental Table 2

Correlations between staining intensities of steroidogenic enzymes and intensities of 18-oxocortisol and 18-hydroxycortisol with regard to all genotypes and *KCNJ5* mutated APAs.

All genotypes (N=136)	CYP11B1		CYP11B2		CYP17A1		HSD3B1		HSD3B2	
	r_s	<i>P</i> -value	r_s	<i>P</i> -value	r_s	<i>P</i> -value	r_s	<i>P</i> -value	r_s	<i>P</i> -value
18-oxocortisol	-0.345	< 0.001	0.086	0.100	-0.171	0.002	-0.040	0.445	0.050	0.367
18-hydroxycortisol	-0.302	< 0.001	0.107	0.039	-0.165	0.002	-0.202	< 0.001	-0.009	0.875

<i>KCNJ5</i> mutated APAs (N=49)	CYP11B1		CYP11B2		CYP17A1		HSD3B1		HSD3B2	
	r_s	<i>P</i> -value	r_s	<i>P</i> -value	r_s	<i>P</i> -value	r_s	<i>P</i> -value	r_s	<i>P</i> -value
18-oxocortisol	-0.421	< 0.001	0.091	0.291	-0.293	< 0.001	0.136	0.117	-0.144	0.130
18-hydroxycortisol	-0.367	< 0.001	-0.040	0.640	-0.345	< 0.001	-0.154	0.075	-0.247	0.009

Supplemental Table 3

Multiple regression analysis for correlations between steroidogenic enzymes and intensities of 18-oxocortisol or 18-hydroxycortisol.

Correlations between steroidogenic enzymes and intensities of 18-oxocortisol

	Unstandardized coefficients		Standardized coefficients		t	P-value
	B	SE	Beta	95% CI		
Constant	0.082	0.044		(-0.004 - 0.168)	1.873	0.062
CYP11B1	-0.699	0.163	-0.253	(-1.019 - -0.378)	-4.282	<0.001
CYP17A1	0.148	0.068	0.128	(0.014 - 0.281)	2.171	0.031

Correlations between steroidogenic enzymes and intensities of 18-hydroxycortisol

	Unstandardized coefficients		Standardized coefficients		t	P-value
	B	SE	Beta	95% CI		
Constant	0.521	0.207		(0.115 - 0.928)	2.523	0.012
CYP11B1	-1.034	0.504	-0.144	(-2.026 - -0.043)	-2.052	0.041
CYP11B2	0.036	0.071	0.032	(-0.104 - 0.176)	0.504	0.615
CYP17A1	0.079	0.190	0.026	(-0.295 - 0.452)	0.414	0.679
HSD3B1	-0.608	0.370	-0.100	(-1.337 - 0.121)	-1.642	0.102

Supplemental Table 4

Correlations between staining intensities of steroidogenic enzymes and intensities of 18-oxocortisol with regard to tissues with lower (low CYP17A1) and higher (high CYP17A1) than median value of staining intensities of CYP17A1.

	18-oxocortisol			
	Tissues with low CYP17A1		Tissues with high CYP17A1	
	r_s	<i>P</i> -value	r_s	<i>P</i> -value
CYP11B1	-0.277	< 0.001	-0.306	< 0.001
CYP11B2	-0.140	0.073	0.104	0.184
CYP17A1	0.213	0.005	-0.226	0.003
HSD3B1	0.032	0.686	-0.048	0.542
HSD3B2	-0.111	0.182	0.057	0.503

Abbreviations SE, standard error; CI, confidence interval. *P*-values of less than 0.05 were considered significant and shown as bolds.

Supplemental Table 5

Relationships between staining intensities of steroidogenic enzymes and clinical outcome.

	Overall	Pairwise comparison					
	<i>P</i> -value	Complete vs partial	Complete vs absent	Partial vs absent	Complete vs partial plus absent	Complete plus partial vs absent	Complete vs absent
CYP11B1	<0.001	0.006	<0.001	0.088	<0.001	0.002	<0.001
CYP11B2	0.449	NA	NA	NA	0.713	0.207	0.324
CYP17A1	0.775	NA	NA	NA	0.549	0.846	0.915
HSD3B1	0.643	NA	NA	NA	0.365	0.627	0.368
HSD3B2	0.037	1.000	0.220	0.043	0.791	0.012	0.074

Staining intensities of three cores from each patient were classified into highest, median and lowest intensity. Median intensity of three cores from each patient were used for immunohistochemistry profiles of steroidogenic enzymes. *P*-values of less than 0.05 were considered significant and shown as bolds. NA = not applicable

Supplemental Table 6

Multivariate logistic regression analyses for clinical success including metabolic profiles.

Complete vs partial plus absent

	Odds ratio (95% CI)	<i>P</i> -value
Age at adrenalectomy (per year)	0.93 (0.88 - 0.98)	0.010
BMI (per 1 kg/m ²)	0.83 (0.72 - 0.95)	0.009
m/z 384.1505 (per 0.01 unit of peak intensity)	1.03 (1.01 - 1.06)	0.005
	Odds ratio (95% CI)	<i>P</i> -value
Age at adrenalectomy (per year)	0.94 (0.89 - 1.00)	0.035
BMI (per 1 kg/m ²)	0.83 (0.72 - 0.95)	0.009
m/z 128.386 (per 0.01 unit of peak intensity)	1.07 (1.02 - 1.12)	0.010
	Odds ratio (95% CI)	<i>P</i> -value
Age at adrenalectomy (per year)	0.93 (0.88 - 0.99)	0.017
BMI (per 1 kg/m ²)	0.81 (0.70 - 0.93)	0.004
m/z 933.5345 (per 0.01 unit of peak intensity)	1.15 (1.03 - 1.27)	0.011
	Odds ratio (95% CI)	<i>P</i> -value
Age at adrenalectomy (per year)	0.91 (0.85 - 0.98)	0.007
BMI (per 1 kg/m ²)	0.80 (0.68 - 0.93)	0.003
Acetohexamide (m/z 345.088) (per 0.01 unit of peak intensity)	0.94 (0.89 - 0.99)	0.012
	Odds ratio (95% CI)	<i>P</i> -value
Age at adrenalectomy (per year)	0.90 (0.85 - 0.97)	0.003
BMI (per 1 kg/m ²)	0.80 (0.69 - 0.94)	0.005
m/z 266.0705 (per 0.01 unit of peak intensity)	0.67 (0.48 - 0.93)	0.015
	Odds ratio (95% CI)	<i>P</i> -value
Age at adrenalectomy (per year)	0.93 (0.88 - 0.99)	0.018
BMI (per 1 kg/m ²)	0.82 (0.71 - 0.95)	0.006
m/z 308.875 (per 0.01 unit of peak intensity)	1.01 (1.00 - 1.01)	0.018

	Odds ratio (95% CI)	<i>P</i> -value
Age at adrenalectomy (per year)	0.93 (0.88 - 0.99)	0.018
BMI (per 1 kg/m ²)	0.82 (0.71 - 0.95)	0.006
m/z 306.876 (per 0.01 unit of peak intensity)	1.01 (1.00 - 1.01)	0.019

	Odds ratio (95% CI)	<i>P</i> -value
Age at adrenalectomy (per year)	0.93 (0.88 - 0.99)	0.017
BMI (per 1 kg/m ²)	0.83 (0.72 - 0.96)	0.011
CDP (m/z 384.0005) (per 0.01 unit of peak intensity)	1.23 (1.02 - 1.49)	0.030

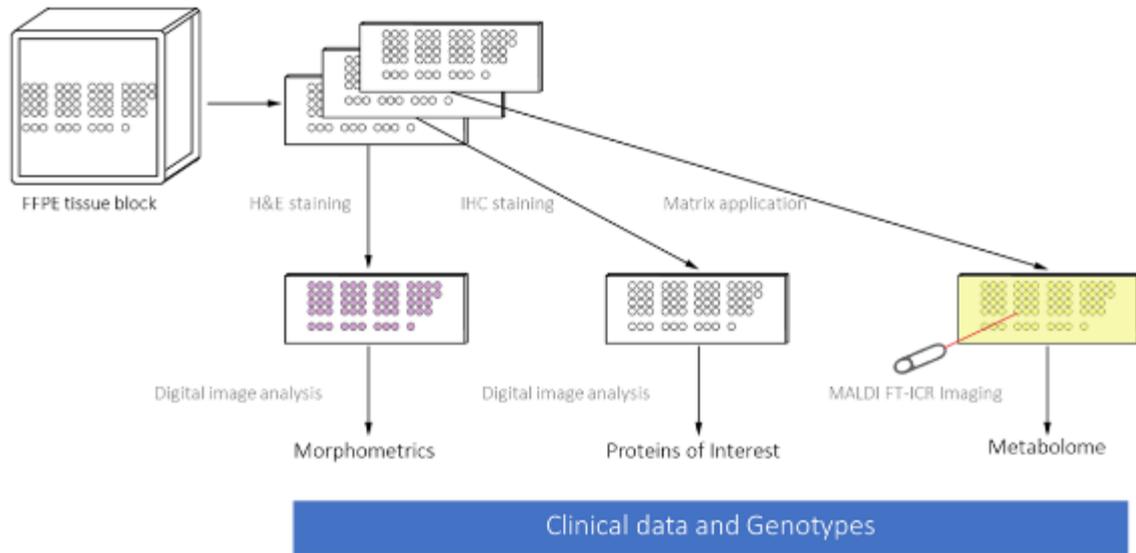
	Odds ratio (95% CI)	<i>P</i> -value
Age at adrenalectomy (per year)	0.95 (0.89 - 1.00)	0.059
BMI (per 1 kg/m ²)	0.79 (0.68 - 0.92)	0.003
m/z 425.2573 (per 0.01 unit of peak intensity)	1.21 (1.01 - 1.45)	0.038

	Odds ratio (95% CI)	<i>P</i> -value
Age at adrenalectomy (per year)	0.93 (0.87 - 0.99)	0.018
BMI (per 1 kg/m ²)	0.81 (0.71 - 0.94)	0.004
m/z 640.525 (per 0.01 unit of peak intensity)	1.07 (1.00 - 1.15)	0.041

P-values of less than 0.05 were considered significant and shown as bolds.

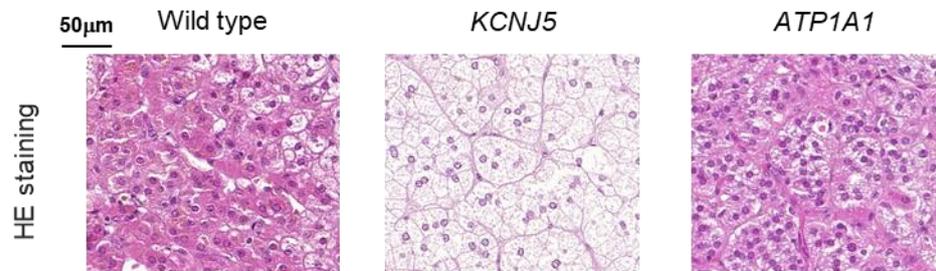
Supplemental Figure 1

Workflow of the project

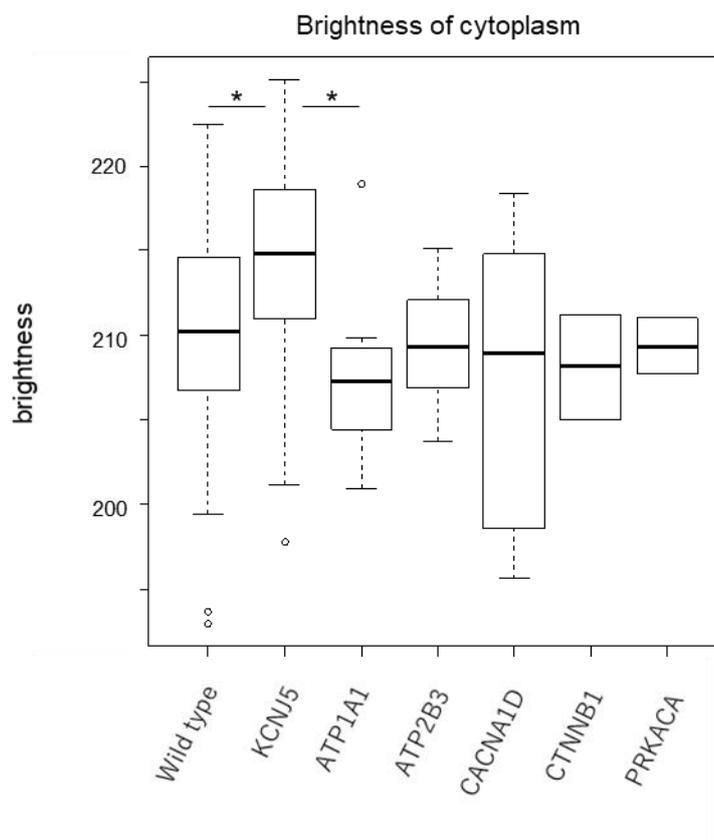


Supplemental Figure 2

A



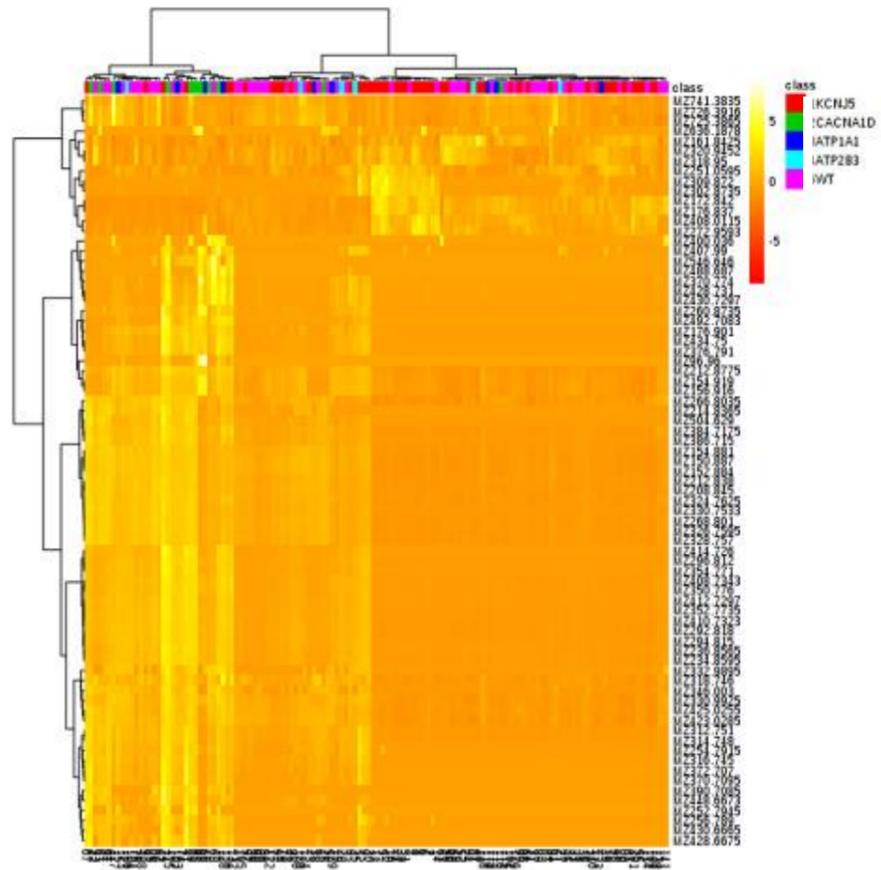
B



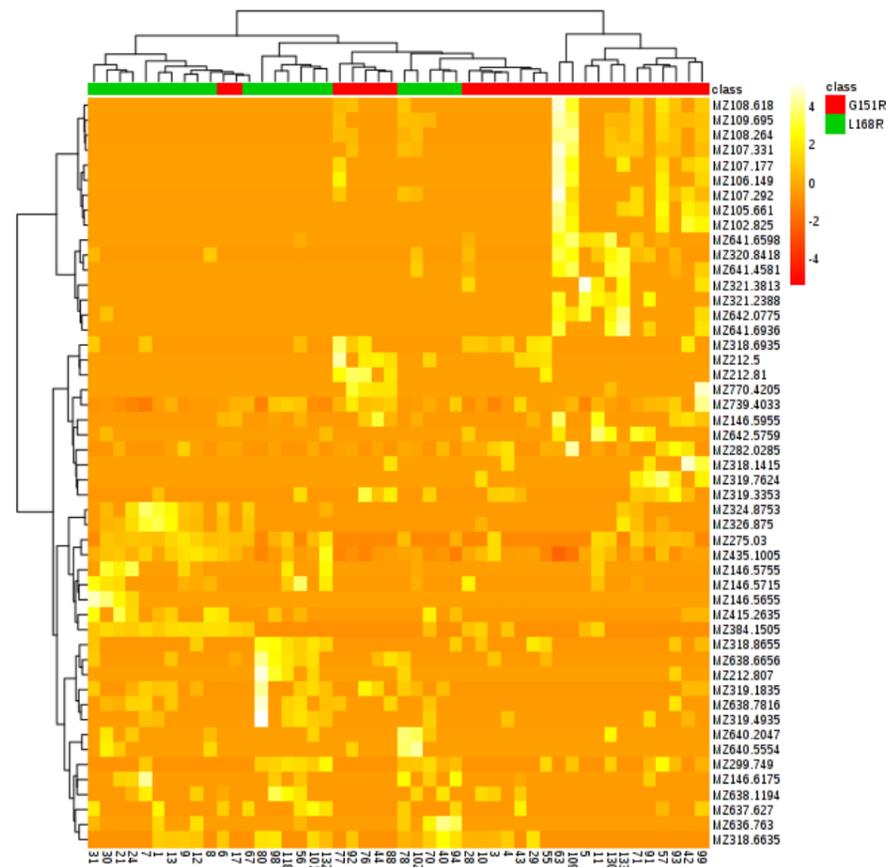
Comparison of morphometric feature and genotypes of 124 APA samples. (A) Representative images of HE staining of Wild type, *KCNJ5* mutated and *ATP1A1* mutated APAs. (B) Brightness of cytoplasm with regard to genotypes (adjusted $P = 0.022$). Kruskal-Wallis test was used for statistical analysis. $*P < 0.05$.

Supplemental Figure 3

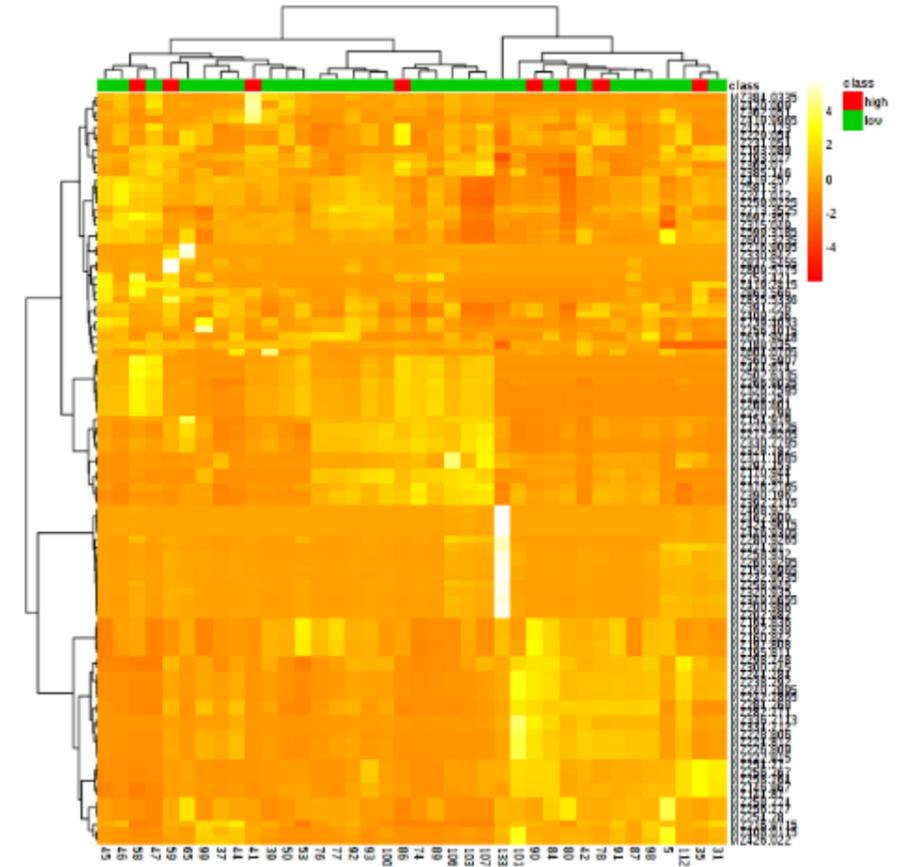
A.



B.

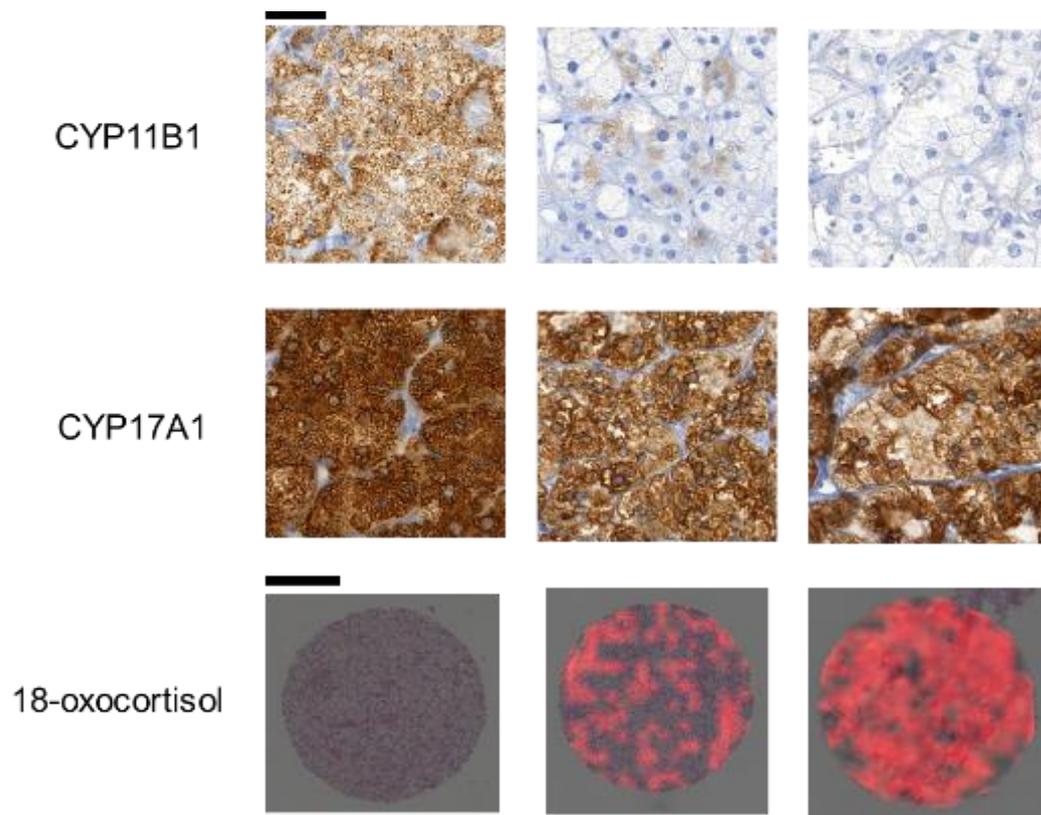


C.



Unguided hierarchical clustering analysis of metabolome profiles among 128 APAs with wildtype, *KCNJ5*, *ATP1A1*, *ATP2B3* and *CACNA1D* mutations (A), those for 48 APAs with *KCNJ5* mutation between G151R and L168R and (B), and those for 38 APAs between high (>1.8 µg/dL) and low (≤1.8 µg/dL) for cortisol after 1mg DST (C).

Supplemental Figure 4



Comparison of immunohistochemistry of CYP11B1 and CYP17A1 and 18-oxocortisol expression (red) in cores with lower than median expression of CYP17A1. Scale bars for metabolites and IHC; 500 and 50 μm , respectively.