

## **SUPPLEMENTARY DATA**

**Supplementary Table 1.** Effects of osilodrostat, metyrapone, and ketoconazole on the steroid profile in human adrenocortical cells

			Progesterone	Corticosterone	17-OHP	11-Deoxycortisol	Cortisol	DHEA	DHEAS	Androstenedione	Testosterone
HAC15	Basal	Osilodrostat 5 µM	+0.7 ± 0.4 (+70%)	-6.4 ± 0.2 (-95%)	-4.6 ± 5.0 (-9%)	-282 ± 144 (-10%)	-79 ± 5.1 (-87%)	-10 ± 1.7 (-38%)	-284 ± 50 (-33%)	-738 ± 118 (-38%)	-11 ± 3.3 (-21%)
		Metyrapone 5 µM	+0.4 ± 0.1 (+31%)	-6.3 ± 0.3 (-95%)	-10 ± 3.1 (-24%****)	-146 ± 73 (-6%)	-77 ± 3.7 (-88%)	-9.5 ± 2.8 (-38%)	-217 ± 35 (-29%)	-500 ± 61 (-31%)	-11 ± 1.6 (-28%)
		Ketoconazole 5 µM	+6.9 ± 0.3 (+644%****)	-3.4 ± 0.1 (-92%)	-33 ± 2.0 (-93%****)	-1539 ± 80 (-79%****)	-65 ± 2.1 (-95%*)	-29 ± 3.5 (-96%****)	-778 ± 72 (-91%****)	-1254 ± 67 (-96%****)	-32 ± 2.3 (-89%****)
	ACTH	Osilodrostat 5 µM	+0.2 ± 0.3 (+4%)	-4.7 ± 1.0 (-84%)	+3.6 ± 2.6 (+10%)	+316 ± 137 (+12%)	-51 ± 4.3 (-87%)	-2.3 ± 2.6 (-8%)	-51 ± 38 (-9%)	-40 ± 89 (-3%)	+0.4 ± 3.1 (+1%)
		Metyrapone 5 µM	-0.6 ± 0.2 (-25%)	-3.9 ± 0.7 (-82%)	-12 ± 1.4 (-28%****)	-398 ± 157 (-13%****)	-50 ± 3.4 (-91%*)	-4.4 ± 1.9 (-19%)	-40 ± 39 (-6%)	-382 ± 112 (-23%****)	-9.6 ± 4.0 (-27%****)
		Ketoconazole 5 µM	+5.6 ± 0.5 (+121%****)	-2.7 ± 0.5 (-75%)	-37 ± 0.9 (-95%****)	-2434 ± 176 (-80%****)	-40 ± 0.8 (-95%****)	-24 ± 2.4 (-91%****)	-452 ± 21 (-88%****)	-1364 ± 93 (-93%****)	-28 ± 3.4 (-85%****)
ACTH-dependent adrenal hyperplasia no. 1	Basal	Osilodrostat 5 µM	+1.9 ± 0.2 (+58%)	-89 ± 7.4 (-98%)	-0.4 ± 1.4 (-2%)	+8.1 ± 32 (+2%)	-200 ± 17 (-99%)	<LLQ	<LLQ	-15 ± 2.2 (-47%)	-0.6 ± 0.3 (-36%)
		Metyrapone 5 µM	+0.2 ± 0.2 (+9%**)	-76 ± 5.2 (-97%)	-8.8 ± 0.6 (-38%****)	-6.9 ± 24 (-2%)	-173 ± 11 (-98%)	<LLQ	<LLQ	-16 ± 0.7 (-56%)	-0.9 ± 0.1 (-61%)
		Ketoconazole 5 µM	+25 ± 0.6 (+655%****)	-144 ± 13 (-96%)	-14 ± 0.9 (-91%****)	-285 ± 6.5 (-98%****)	-171 ± 14 (-98%)	<LLQ	<LLQ	-8.3 ± 0.5 (-94%****)	-0.3 ± 0.1 (-61%)
	ACTH	Osilodrostat 5 µM	+2.3 ± 0.3 (+82%)	-241 ± 5.7 (-98%)	-3.5 ± 6.1 (-7%)	+347 ± 144 (+59%)	-845 ± 15 (-99%)	<LLQ	<LLQ	-17 ± 2.4 (-33%)	-0.3 ± 0.2 (-18%)
		Metyrapone 5 µM	+0.9 ± 0.2 (+44%)	-76 ± 5.2 (-97%)	-11 ± 3.8 (-27%)	+557 ± 86 (+90%)	-894 ± 32 (-99%)	<LLQ	<LLQ	-14 ± 2.5 (-31%)	-0.8 ± 0.2 (-44%)
		Ketoconazole 5 µM	+60 ± 1.4 (+999%****)	-445 ± 16 (-99%)	-32 ± 3.1 (-85%****)	-469 ± 13 (-100%****)	-842 ± 24 (-100%)	<LLQ	<LLQ	-19 ± 1.0 (-97%****)	-0.6 ± 0.1 (-84%***)
ACTH-dependent adrenal hyperplasia no. 2	ACTH	Osilodrostat 0.1 µM	< LLQ	-22 ± 4.8 (-37%)	+0.05 ± 0.1 (+2%)	+83 ± 2.5 (+278%)	-194 ± 23 (-44%)	<LLQ	<LLQ	+6.5 ± 0.4 (+205%)	+0.1 ± 0.0 (+271%)
		Metyrapone 0.1 µM	< LLQ	-17 ± 4.5 (-28%)	+0.5 ± 0.1 (+21%*)	+90 ± 2.3 (+339%)	-158 ± 11 (-36%)	<LLQ	<LLQ	+6.3 ± 0.4 (+229%)	+0.2 ± 0.0 (+403%)

	Ketoconazole 0.1 $\mu$ M	< LLQ	<b>+91 <math>\pm</math> 4.1</b> (+124%****)	<b>+0.3 <math>\pm</math> 0.1</b> (+15%)	+0.4 $\pm$ 1.1 (+1725%****)	<b>-99 <math>\pm</math> 40</b> (-16%****)	< LLQ	< LLQ	<b>-0.4 <math>\pm</math> 0.1</b> (-22%****)	< LLQ
	Osilodrostat 0.5 $\mu$ M	< LLQ	<b>-44 <math>\pm</math> 4.1</b> (-74%)	-0.1 $\pm$ 0.2 (-5%)	<b>+154 <math>\pm</math> 6.1</b> (+513%)	<b>-371 <math>\pm</math> 13</b> (-83%)	< LLQ	< LLQ	<b>+8.5 <math>\pm</math> 0.7</b> (+268%)	<b>+0.2 <math>\pm</math> 0.0</b> (+348%)
	Metyrapone 0.5 $\mu$ M	< LLQ	<b>-40 <math>\pm</math> 4.3</b> (-67%)	<b>+1.1 <math>\pm</math> 0.1</b> (+46%****)	<b>+221 <math>\pm</math> 6.1</b> (+837%****)	<b>-333 <math>\pm</math> 7.8</b> (-77%)	< LLQ	< LLQ	<b>+11 <math>\pm</math> 0.4</b> (+411%****)	<b>+0.2 <math>\pm</math> 0.0</b> (+477%)
	Ketoconazole 0.5 $\mu$ M	< LLQ	<b>+183 <math>\pm</math> 4.5</b> (+250%****)	<b>+0.6 <math>\pm</math> 0.1</b> (+30%****)	<b>+1.9 <math>\pm</math> 1.1</b> (+9450****)	<b>-371 <math>\pm</math> 40</b> (-60%****)	< LLQ	< LLQ	<b>-1.3 <math>\pm</math> 0.1</b> (-72%****)	< LLQ
	Osilodrostat 5 $\mu$ M	< LLQ	<b>-55 <math>\pm</math> 4.2</b> (-93%)	+0.1 $\pm$ 0.1 (+5%)	<b>+218 <math>\pm</math> 5.7</b> (+728%)	<b>-427 <math>\pm</math> 13</b> (-96%)	< LLQ	< LLQ	<b>+7.2 <math>\pm</math> 0.4</b> (+228%)	<b>+0.2 <math>\pm</math> 0.0</b> (+324%)
	Metyrapone 5 $\mu$ M	< LLQ	<b>-56 <math>\pm</math> 4.3</b> (-95%)	<b>+0.9 <math>\pm</math> 0.1</b> (+36%****)	<b>+287 <math>\pm</math> 5.8</b> (+1086%****)	<b>-421 <math>\pm</math> 8.0</b> (-97%)	< LLQ	< LLQ	<b>+6.7 <math>\pm</math> 0.5</b> (+245%)	<b>+0.1 <math>\pm</math> 0.0</b> (+317%)
	Ketoconazole 5 $\mu$ M	< LLQ	<b>-60 <math>\pm</math> 3.4</b> (-82%)	+0.2 $\pm$ 0.1 (+10%)	<b>-8.7 <math>\pm</math> 1.1</b> (-44%****)	<b>-617 <math>\pm</math> 39</b> (-99%)	< LLQ	< LLQ	<b>-1.7 <math>\pm</math> 0.1</b> (-95%****)	< LLQ

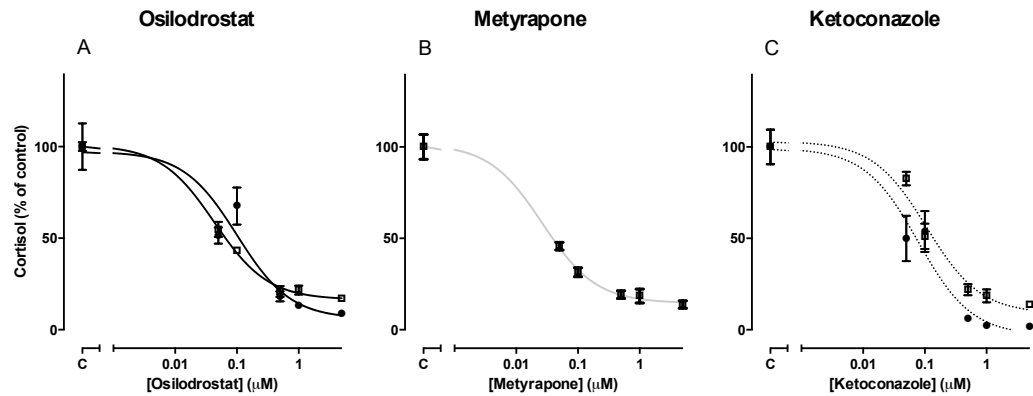
Effects of osilodrostat, metyrapone, and ketoconazole on levels of progesterone, corticosterone, 17-hydroxyprogesterone (17-OHP), 11-deoxycortisol, cortisol, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione, and testosterone in HAC15 cells and two ACTH-dependent adrenal hyperplasias. Numbers of the primary cultures correspond with the numbers in Table 1 and 2. Data are presented as absolute concentration (nmol/L) change  $\pm$  standard error of the difference compared to vehicle treated control (basal) or compared to ACTH stimulation (ACTH), with percentage change compared to control between brackets. Significant absolute changes compared to control are depicted in bold. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and \*\*\*\*  $P < 0.0001$  compared to the percentage change by osilodrostat. ACTH, adrenocorticotropic hormone; LLQ, lower limit of quantitation.

**Supplementary Table 2.** Effects of osilodrostat, metyrapone, and ketoconazole on the steroid profile in two primary human adrenocortical cell cultures

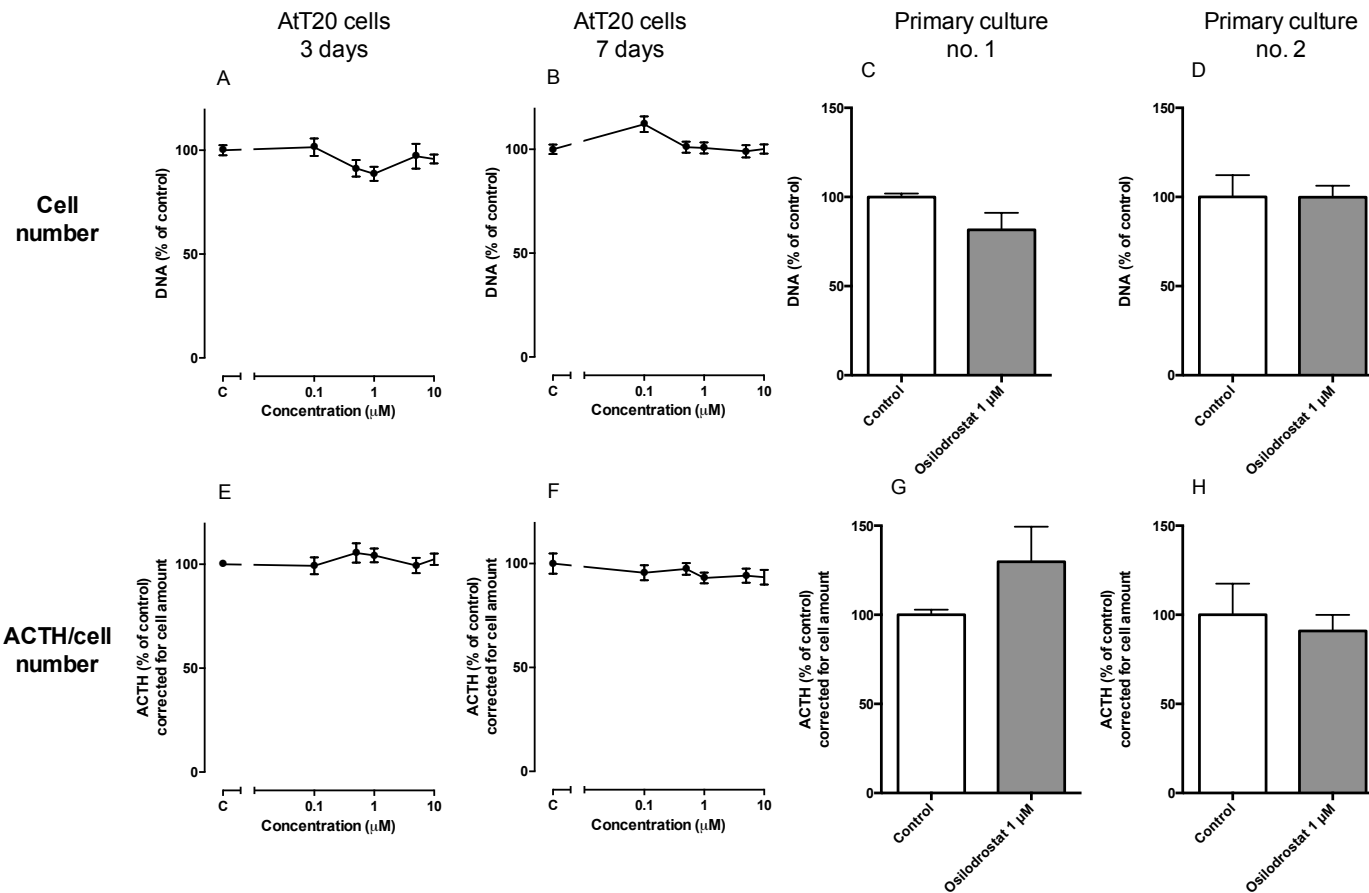
			Progesterone	Corticosterone	17-OHP	11-Deoxycortisol	Cortisol	DHEA	DHEAS	Androstenedione	Testosterone
<b>Cortisol-producing ACA no. 1</b>	<b>Basal</b>	Osilodrostat 1 $\mu$ M	+0.1 $\pm$ 0.1 (+10%)	<b>-10 <math>\pm</math> 0.9</b> (-67%)	+0.7 $\pm$ 0.5 (+6%)	<b>+105 <math>\pm</math> 13</b> (+100%)	<b>-49 <math>\pm</math> 4.0</b> (-80%)	+0.0 $\pm$ 0.1 (+0%)	<LLQ	<b>+4.3 <math>\pm</math> 0.8</b> (+36%)	<b>+0.2 <math>\pm</math> 0.0</b> (+41%)
		Ketoconazole 1 $\mu$ M	<b>+2.1 <math>\pm</math> 0.3</b> (+263%****)	<b>+23 <math>\pm</math> 6.7</b> (-68%****)	<b>+1.9 <math>\pm</math> 0.3</b> (+22%*)	-6.4 $\pm$ 7.2 (-6%****)	<b>-67 <math>\pm</math> 23</b> (-47%)	<b>-1.6 <math>\pm</math> 0.1</b> (-60%****)	<LLQ	<b>-6.0 <math>\pm</math> 0.5</b> (-71%****)	<b>-0.2 <math>\pm</math> 0.0</b> (-47%****)
	<b>ACTH</b>	Osilodrostat 1 $\mu$ M	+0.2 $\pm$ 0. (+8%)	<b>-126 <math>\pm</math> 17</b> (-85%)	-0.6 $\pm$ 6.1 (-1%)	<b>+600 <math>\pm</math> 83</b> (+117%)	<b>-734 <math>\pm</math> 114</b> (-90%)	<LLQ	<LLQ	<b>+17 <math>\pm</math> 5.8</b> (+43%)	+0.1 $\pm$ 0.2 (+6%)
		Ketoconazole 1 $\mu$ M	<b>+4.7 <math>\pm</math> 2.3</b> (+238%***)	<b>+131 <math>\pm</math> 51</b> (+65%****)	+3.0 $\pm$ 1.7 (+10%)	+36 $\pm$ 16 (+13%****)	-276 $\pm$ 155 (-90%)	<LLQ	<LLQ	<b>-7.7 <math>\pm</math> 3.0</b> (-56%***)	-0.2 $\pm$ 0.1 (-46%)
<b>Cortisol-producing ACA no. 2</b>	<b>Basal</b>	Osilodrostat 1 $\mu$ M	+0.1 $\pm$ 0.1 (+13%)	<b>-36 <math>\pm</math> 4.9</b> (-37%)	-0.8 $\pm$ 3.7 (-1%)	<b>+527 <math>\pm</math> 23</b> (+202%)	<b>-630 <math>\pm</math> 34</b> (-75%)	-0.5 $\pm$ 0.4 (-9%)	<LLQ	+22 $\pm$ 11 (+18%)	<b>+2.0 <math>\pm</math> 0.6</b> (+28%)
		Metyrapone 1 $\mu$ M	<b>-0.1 <math>\pm</math> 0.0</b> (-19%)	<b>-36 <math>\pm</math> 3.8</b> (-42%)	<b>-16 <math>\pm</math> 2.1</b> (-19%*)	<b>+454 <math>\pm</math> 16</b> (+158%***)	<b>-704 <math>\pm</math> 11</b> (-87%)	<b>-0.7 <math>\pm</math> 0.2</b> (-11%)	<LLQ	<b>+19 <math>\pm</math> 6.3</b> (+13%)	<b>+1.2 <math>\pm</math> 0.5</b> (+13%)
		Ketoconazole 1 $\mu$ M	<b>+0.7 <math>\pm</math> 0.1</b> (+77%***)	<b>+125 <math>\pm</math> 26</b> (+92%****)	<b>-27 <math>\pm</math> 3.4</b> (-54%****)	<b>-143 <math>\pm</math> 26</b> (-41%****)	<b>-430 <math>\pm</math> 60</b> (-38%****)	<b>-3.9 <math>\pm</math> 0.3</b> (-65%****)	<LLQ	<b>-88 <math>\pm</math> 2.7</b> (-82%****)	<b>-6.6 <math>\pm</math> 0.2</b> (-80%****)
	<b>ACTH</b>	Osilodrostat 1 $\mu$ M	<b>+0.0 <math>\pm</math> 0.0</b> (+21%)	<b>-483 <math>\pm</math> 39</b> (-53%)	+7.8 $\pm$ 1.2 (+38%)	<b>+2944 <math>\pm</math> 103</b> (+2022%)	<b>-2908 <math>\pm</math> 310</b> (-51%)	<b>+1.3 <math>\pm</math> 0.3</b> (+22%)	<LLQ	<b>+369 <math>\pm</math> 14</b> (+331%)	<b>+21 <math>\pm</math> 0.4</b> (+382%)
		Metyrapone 1 $\mu$ M	+0.0 $\pm$ 0.0 (+8%)	<b>-741 <math>\pm</math> 10</b> (-78%**)	<b>+14 <math>\pm</math> 0.9</b> (+75%****)	<b>+3838 <math>\pm</math> 116</b> (+2993****)	<b>-4495 <math>\pm</math> 90</b> (-77%****)	<b>+1.5 <math>\pm</math> 0.3</b> (+26%)	<LLQ	<b>+415 <math>\pm</math> 13</b> (+404%****)	<b>+22 <math>\pm</math> 1.0</b> (+525%****)
		Ketoconazole 1 $\mu$ M	<b>+0.1 <math>\pm</math> 0.0</b> (+50%*)	<b>+1245 <math>\pm</math> 86.</b> (+137%****)	<b>-3.3 <math>\pm</math> 0.7</b> (-21%****)	<b>-24 <math>\pm</math> 8.0</b> (-24%****)	<b>-2660 <math>\pm</math> 244</b> (-39%)	-1.0 $\pm$ 0.4 (-24%)	<LLQ	<b>-29 <math>\pm</math> 2.7</b> (-64%****)	<b>-1.3 <math>\pm</math> 0.2</b> (-66%****)

Effects of osilodrostat, metyrapone, and ketoconazole on levels of progesterone, corticosterone, 17-hydroxyprogesterone (17-OHP), 11-deoxycortisol, cortisol, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione, and testosterone, in two primary cortisol-producing adrenal adenoma cultures. Numbers of the primary cultures correspond with the numbers in Table 1 and 2. Data are presented as absolute concentration (nmol/L) change  $\pm$  standard error of the difference compared to vehicle treated control (basal) or compared to ACTH stimulation (ACTH), with percentage change compared to control between brackets. Significant absolute changes compared to control are depicted in bold. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and \*\*\*\*  $P < 0.0001$  compared to the percentage change by osilodrostat. ACA, adrenocortical adenoma; ACTH, adrenocorticotrophic hormone; LLQ, lower limit of quantitation.

### Adrenocortical carcinoma



**Supplementary Figure 1.** Effects of osilodrostat (left, black solid lines), metyrapone (middle, grey solid line), and ketoconazole (right, black dotted lines) on basal cortisol production in primary human adrenocortical carcinoma cultures. The different concentrations that are tested are 0.01, 0.05, 0.1, 0.5, 1 and 5  $\mu\text{M}$ , but not all concentrations were tested in every primary culture. Symbols are presented in Table 2. Values are depicted as mean  $\pm$  SEM and as percentage of vehicle treated control. C, control.



**Supplementary Figure 2.** Effects of osilodrostat on cell amount (upper row, A-D) and ACTH secretion corrected for cell amount (bottom row, E-H) in mouse pituitary AtT20 cells (A-B, E-F) and in two primary human corticotroph pituitary adenoma cultures (C-D, G-H). Primary cultures were incubated with treatment for 7 days. Values are depicted as mean  $\pm$  SEM and as percentage of vehicle treated control.