

## **SUPPLEMENTARY MATERIAL**

### **Supplementary Information 1**

#### ***ABC***

Time- and concentration-dependent reductions in total cell protein and lactate were seen in cultures of RPT cells and skeletal muscle cells treated with ABC (Supplementary Table S1); although for skeletal muscle cells, this was only significant at the higher 200  $\mu\text{M}$  concentration. A similar effect was observed for adipocytes, although decreases in lactate did not appear to be time- or concentration-dependent and a significant decrease in total cell protein was only seen at Day 9 and not maintained at later time points. Significant decreases in cellular ATP levels compared with controls were seen for both concentrations in adipocytes and by Day 19 in RPT cells treated with 200  $\mu\text{M}$  ABC. Conversely, at the lower 23  $\mu\text{M}$  concentration, ATP levels in RPT cells significantly increased relative to control cultures. ATP levels in skeletal muscle cells significantly increased at both concentrations of ABC.

#### ***AZT***

Significant time- and concentration-related decreases in cell protein were observed in RPT cultures exposed to AZT (Supplementary Table S1). Significant reductions in skeletal muscle myoblast cell protein were observed at the 200  $\mu\text{M}$  AZT concentration only. Similar cytotoxicity was not observed in differentiated adipocytes; however, there was an approximately 20% reduction in cell ATP content by Day 19 in adipocytes treated with 200  $\mu\text{M}$  AZT. In contrast AZT induced significant stress-related increases in ATP content in RPT and skeletal muscle cell cultures, with the exception of RPT cells exposed to the higher 200  $\mu\text{M}$  concentration, which experienced either no change or a significant reduction (Day 19). Significant reductions in lactate were seen in all cultures exposed to 200  $\mu\text{M}$  AZT.

Significant reductions in mtDNA were only observed in RPT cultures exposed to 200  $\mu\text{M}$  AZT. At the lower concentration, AZT induced increases in RPT cell mtDNA and trends towards an increase in mtDNA were observed in skeletal muscle myoblasts. No significant changes were observed in differentiated adipocytes.

**SUPPLEMENTARY TABLE S1** Mitochondrial DNA content and viability measures in primary cultures of human renal proximal tubule cells, differentiated adipocytes and skeletal muscle myoblasts exposed to ABC and AZT

		Mean ± SE											
Control		mtDNA ATP8, %			Protein, µg/mL			ATP, LU x 10 <sup>3</sup> /µg protein			Lactate, µg/mg protein		
Cell type	Concn, µM	Day 9	Day 14	Day 19	Day 9	Day 14	Day 19	Day 9	Day 14	Day 19	Day 9	Day 14	Day 19
Human renal proximal tubule cells	—	100	100	100	192.3±6.9	122.3±3.3	94.7±2.6	166.6±4.1	223.7±1.4	226.2±2.0	1.8±0.1	0.8±0.0	0.6±0.0
Human differentiated adipocytes	—	100	100	100	372.2±4.4	296.9±3.2	387.7±23.7	67±1	99±3	78±2	0.47±0.0 <sub>1</sub>	0.55±0.0 <sub>3</sub>	0.75±0.0 <sub>2</sub>
Human skeletal muscle myoblasts	—	100	100	100	753.6±78.3	580.8±37.1	648.7±50.9	6.5±0.5	7.0±0.6	5.6±0.2	1.7±0.0	1.7±0.0	2.6±0.1
		Mean percentage versus control ± SE											
ABC		mtDNA ATP8			Protein			ATP			Lactate		
Cell type	Concn, µM	Day 9	Day 14	Day 19	Day 9	Day 14	Day 19	Day 9	Day 14	Day 19	Day 9	Day 14	Day 19
Human renal proximal tubule cells	23	108±5	138±5.5	62.4±3.9	84.9±3.2	62.1±9.7	46.4±7.7	111.9±0.5	138±2	146.5±2.1	81.2±0.6	78.2±2.9	71.6±1.9
	200	52.2±5.5	99.7±12.4	42.3±11.6	42.4±2.5	16.7±18.8	4.7±19.1	99.2±0.8	91.3±2.9	59.3±1.8	32.1 ± 2.3	14.6 ± 3.3	7.0 ± 4.5

Human differentiated adipocytes	23	141±7.5	134±14.8	146±6.3	77.5±0.7	98.4±2.7	82.2±7.7	85.1±2.2	75.5±2.1	108.5±1.1	93.5±1.9	86.4±4.1	64.1±3.5
	200	150.4±16.5	116.4±6.2	100.4±7.2	80.6±1.0	93±1.6	90.7±7.2	69.5±3.3	62.8±1.7	70±4.2	69.5±3.3	62.8±1.7	70±4.2
Human skeletal muscle myoblasts	23	99.7±7.7	90.6±3.7	89.7±6.7	73.2±5.5	65.1±2.8	74.9±9.2	181.3±5.8	205.2±8.9	139.7±8.1	106±3.8	105.1±1.8	92.2±2.4
	200	112±11.2	87.4±10.7	51.3±3.8	52.7±1.7	51±2.3	47.9±4.6	212.6±6.1	295.5±3.8	309.1±2.8	53±1.5	58.7±2.5	46.8±2.8
<b>Mean percentage versus control ± SE</b>													
<b>AZT</b>		<b>mtDNA ATP8</b>			<b>Protein</b>			<b>ATP</b>			<b>Lactate</b>		
<b>Cell type</b>	<b>Concn, <math>\mu</math>M</b>	<b>Day 9</b>	<b>Day 14</b>	<b>Day 19</b>	<b>Day 9</b>	<b>Day 14</b>	<b>Day 19</b>	<b>Day 9</b>	<b>Day 14</b>	<b>Day 19</b>	<b>Day 9</b>	<b>Day 14</b>	<b>Day 19</b>
Human renal proximal tubule cells	14.5	94.3±6	154±7.6	120±13.1	84.8±2.3	67.8±3.9	29.4±28.3	110.5±0.5	126.9±1.6	232.9±1.1	97.3±1.4	92.3±4.8	89.1±1
	200	59.7±2.3	84.7±7.5	58.2±5.9	81.4±3.1	65.5±12.8	58.2±34	99.4±1.2	98.8±8.1	58.9±5.1	69±2	48.9±4.3	39.6±7
Human differentiated adipocytes	14.5	116±5.5	109±10.4	91.6±4.7	93.3±4.3	103.2±4.1	103.7±5.5	100.5±6	85.8±3.1	94±3.5	93.3±2.7	73.1±6.7	81.1±3
	200	137.3±13.9	104.7±4.5	82.8±5.8	94.6±1	105±1.1	104±4.5	96.7±1.8	83±3.1	77.7±5	85.6±4.1	78.2±6.6	72.7±3.6
Human skeletal muscle myoblasts	14.5	90.7±10.8	119±2.7	173±40.9	78.9±10.4	81.2±2.3	101.1±10.4	149.1±10.4	91.7±11.6	88.1±8.1	107.1±4.6	104.3±1.7	96.7±3.6
	200	142±4.9	71.4±5.7	114±6.9	63.4±3.8	80.6±3.4	71.6±4.8	179.7±10.6	140.6±8	172.4±3.8	84.6±3.1	89.5±3.2	80.2±0.1

All experiments were performed in triplicate

Dark gray cells represent significant increase versus control ( $P < 0.05$ ); light gray cells represent significant decrease versus control ( $P < 0.05$ )

ABC, abacavir; AZT, azidothymidine; LU, light intensity unit; mtDNA, mitochondrial DNA; SE, standard error