

## **Drug Metabolism and Disposition**

### **Supplemental Data**

#### **Differential Selectivity of Human and Mouse ABCC4/Abcc4 for Arsenic Metabolites**

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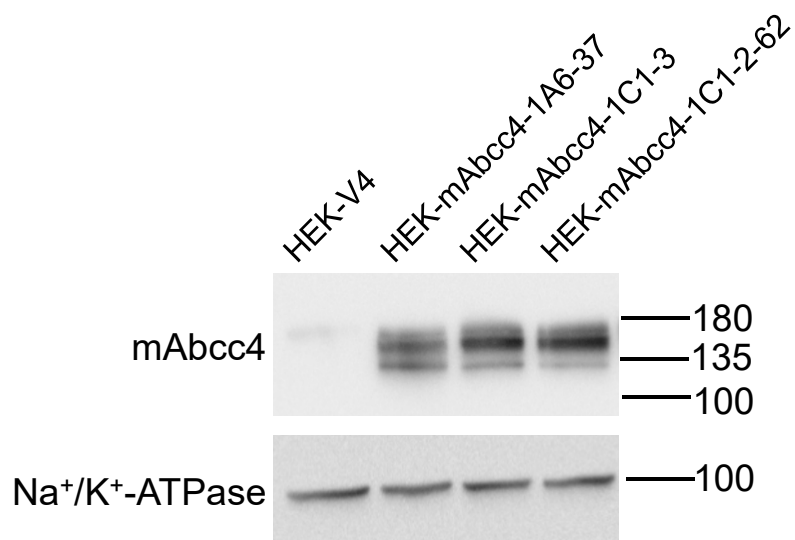
**Supplemental Figure 1:** Immunoblot of whole cell lysates prepared from three independent HEK293 cells expressing mAbcc4 (HEK-mAbcc4-1A6-37, -1C1-3, and 1C1-2-62) and empty vector (HEK-V4).

**Supplemental Figure 2:** Time course and concentration dependence of 17 $\beta$ -estradiol 17-( $\beta$ -D-glucuronide) transport by mAbcc4- and hABCC4-enriched membrane vesicles prepared from *Spodoptera frugiperda* (SF) 21 cells.

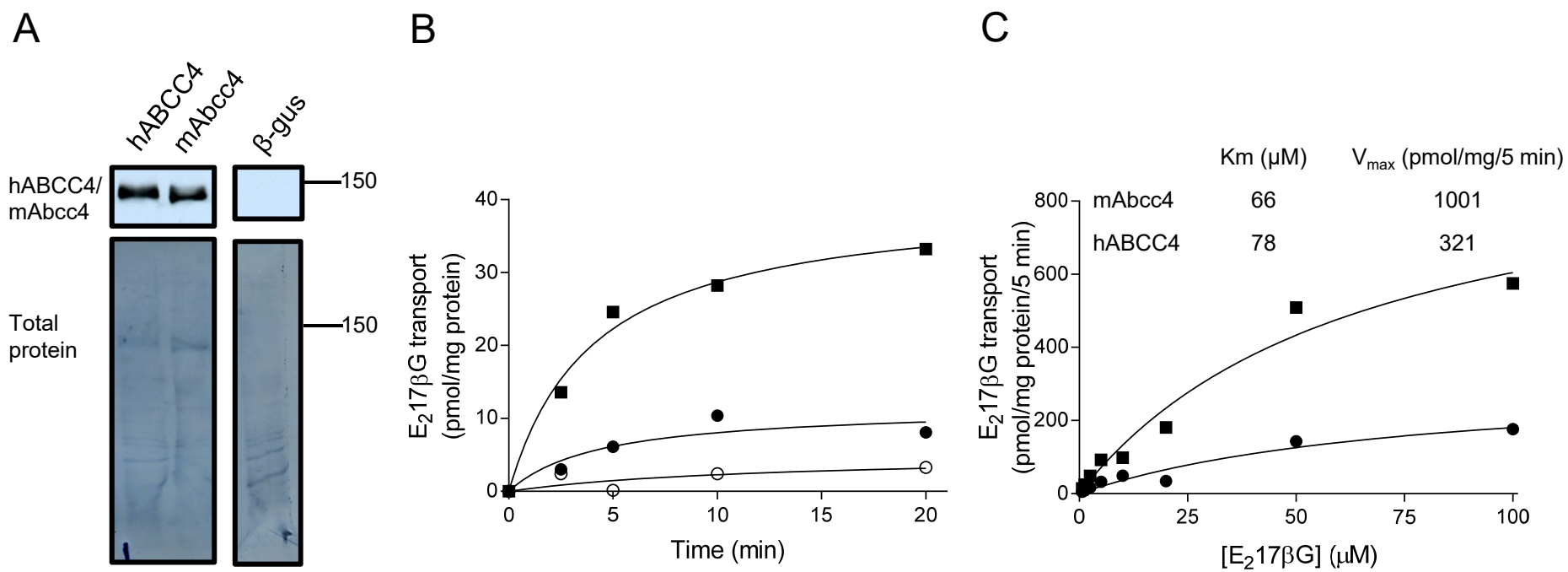
**Supplemental Figure 3:** Raw immunoblot data for Figure 3A.

**Supplemental Table 1:** Relative resistance of HEK293 cells stably expressing mAbcc4 (independent clone HEK-mAbcc4-1C1-262) or empty vector (HEK-V4) to inorganic and methylated arsenic species.

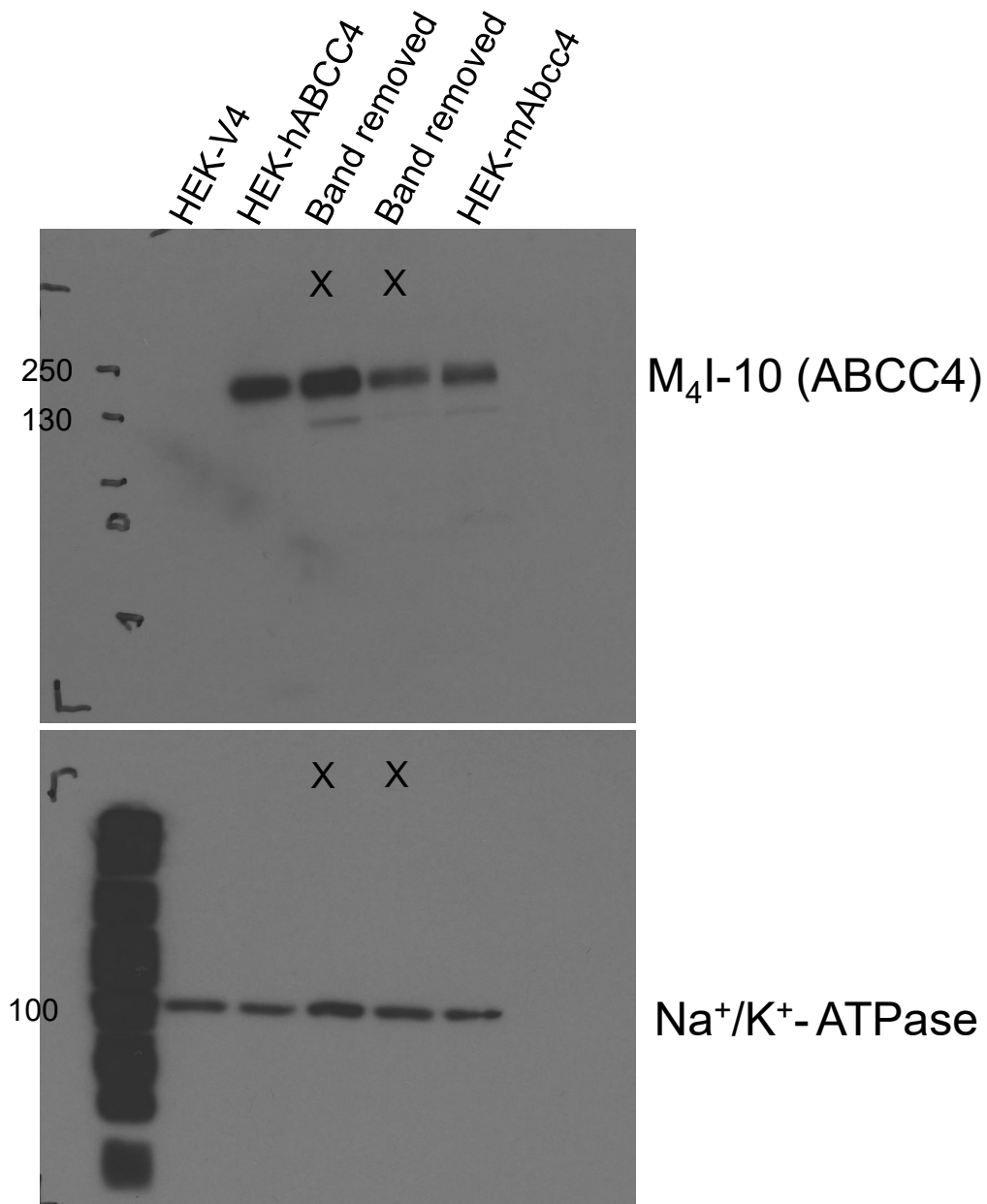
**Supplemental Table 2:** Relative resistance of HEK293 cells stably expressing mAbcc4 (independent clone HEK-mAbcc4-1C1-3) or empty vector (HEK-V4) to inorganic and methylated arsenic species.



**Supplemental Figure 1:** Immunoblot of whole cell lysates prepared from three independent HEK293 cells stably expressing mAbcc4 and empty vector. Whole cell lysates (15  $\mu$ g protein per lane) prepared from stable clonal cell lines HEK-V4 (empty vector), HEK-mAbcc4-1A6-37, -mAbcc4-1C1-3, and mAbcc4-1C1-2-62 were resolved on 6% SDS-PAGE and then electrotransferred to a PVDF membrane. Relative levels of mAbcc4 in whole cell lysates were determined by immunoblotting with mAb M4I-10 (1:2000). Blots were then stripped and probed with anti-Na<sup>+</sup>/K<sup>+</sup>-ATPase mouse MAb (H-3) (1:10,000) as a loading control. Shown is a representative blot and a similar result was obtained in one additional experiment.



**Supplemental Figure 2:** Time course and concentration dependence of 17 $\beta$ -estradiol 17-( $\beta$ -D-glucuronide) transport by mAbcc4- and hABCC4-enriched membrane vesicles prepared from *Spodoptera frugiperda* (SF21) cells. Recombinant baculovirus production of full-length hABCC4, mAbcc4, and  $\beta$ -glucuronidase ( $\beta$ -gus, negative control) and viral infection were completed as previously described (Iram and Cole, 2014). Membrane vesicles were prepared as described in Methods for HEK293 cells. A) hABCC4-, mAbcc4-, and  $\beta$ -gus SF21 membrane vesicles (2  $\mu$ g protein) were resolved by SDS-PAGE and then electrotransferred to a PVDF membrane. The blot was then probed with mAb M<sub>4</sub>I-10 (1:1000) (top panel) and subsequently stained for total protein with amido black (bottom panel). B, C) The transport of 17 $\beta$ -estradiol 17-( $\beta$ -D-glucuronide) (E<sub>2</sub>17 $\beta$ G) by mAbcc4 (■), hABCC4 (●) and  $\beta$ -gus (○) enriched membrane vesicles (5  $\mu$ g protein) was measured at 37°C, as described for HEK293 cells in Methods. Symbols represent mean values of duplicate determinations in a single experiment. B) Time course of E<sub>2</sub>17 $\beta$ G (1  $\mu$ M; 60 nCi) transport. C) Concentration dependence of E<sub>2</sub>17 $\beta$ G transport (0.6-100  $\mu$ M; 60-120 nCi).



**Supplemental Figure 3:** Raw immunoblot data from Figure 3A. Relative levels of mAbcc4 and hABCC4 in whole cell lysates (10  $\mu$ g protein per lane) prepared from stable clonal cell lines HEK-V4 (empty vector), HEK-hABCC4, and HEK-mAbcc4-1A6-37 were determined by immunoblotting with mAb M4I-10 (1:10000) (top). Blots were then probed with anti- $Na^+/K^+-ATPase$  pAb (H-300) (1:2000) as a loading control (bottom).

**Supplemental Table 1:** Relative resistance (RR) of HEK293 cells stably expressing mAbcc4 (independent clone HEK-mAbcc4-1C1-262) or empty vector (HEK-V4) to inorganic and methylated arsenic species.

Chemical	N	EC <sub>50</sub> (± SD) (μM)		RR <sup>a</sup>
		HEK-V4	HEK-mAbcc4-1C1-262	Mouse
As <sup>III</sup>	4	6.4 ± 1.1	4.2 ± 1.3	0.6*
As <sup>V</sup>	4	49.0 ± 5.0	40.0 ± 4.8	0.8*
MMA <sup>III</sup>	5	2.4 ± 0.9	1.7 ± 0.9	0.7
DMA <sup>III</sup>	4	1.4 ± 0.4	1.2 ± 0.4	1.1
6-MP	5	3.2 ± 1.0	144 ± 64	45*

<sup>a</sup>Ratio of EC<sub>50</sub> HEK-mAbcc4/ EC<sub>50</sub> HEK-V4 (vector control)

\*EC<sub>50</sub> for HEK-mAbcc4 or HEK-hAbcc4 is significantly different from EC<sub>50</sub> for HEK-V4 (vector control), *P* < 0.05 (Student's t-test).

**Supplemental Table 2:** Relative resistance (RR) of HEK293 cells stably expressing mAbcc4 (independent clone HEK-mAbcc4-1C1-3) or empty vector (HEK-V4) to inorganic and methylated arsenic species.

Chemical	N	EC <sub>50</sub> (± SD) (μM)		RR <sup>a</sup>
		HEK-V4	HEK-mAbcc4-1C1-3	Mouse
As <sup>III</sup>	2	5.8, 5.3	7.0, 3.8	1.2, 0.7
As <sup>V</sup>	2	55, 55	61, 53	1.1, 1.0
MMA <sup>III</sup>	3	3.0 ± 0.3	1.7 ± 0.5	0.6
DMA <sup>III</sup>	4	1.9 ± 0.5	2.0 ± 1.0	1.1
6-MP	4	2.3 ± 0.8	112 ± 32	48*

<sup>a</sup>Ratio of EC<sub>50</sub> HEK-mAbcc4/ EC<sub>50</sub> HEK-V4 (vector control)

\*EC<sub>50</sub> for HEK-mAbcc4 or HEK-hAbcc4 is significantly different from EC<sub>50</sub> for HEK-V4, *P* < 0.05 (Student's t-test).