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The diamidine diminazene aceturate is a substrate for the High Affinity Pentamidine Transporter: implications for the development of high resistance levels in trypanosomes.

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Supplementary material

Results

Verification of the identity of the tbat1$^{-/-}$ cell line by PCR of resistance markers.

To confirm the identity of the *tbat1*$^{-/-}$ cell line prior to adaptation with diminazene aceturate, DNA extracted from an FTA® card (Whatman) spotted with a bloodstream form culture was PCR amplified for the neomycin gene, which is one of the markers that was used in the plasmid construction of the *tbat1*$^{-/-}$ cell line (Matovu et al., 2003). Amplification of the *TbAT1* gene was also carried out as described (Kazibwe et al., 2009). The *TbAT1* knock-out cell line was also tested against neomycin in culture. Wild type *T. brucei* (s427) with TbAT1/P2 activity was included in each test as a control. Amplification of the 800 bp size fragment of the neomycin gene (Fig. S2,

panel A) and lack of amplification of the 1400 bp fragment which corresponds to the *TbAT1* gene open reading frame (Fig. S2, panel B), confirmed the identity of the cell line. The cell line was also resistant to 1.5 µg/ml of neomycin in culture, unlike wild type *T. brucei* which was susceptible, thus further confirming its identity.