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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 Figure legends

Figure S1. Transport of 1  $\mu\text{M}$  [ $^3\text{H}$ ]-pentamidine by *T. b. brucei* s427WT procyclic forms, in the presence (□) or absence (■) of 1 mM unlabelled pentamidine. The experiment was performed in triplicate and error bars indicate SEM; when not shown fall within the symbol.

Figure S2. PCR amplification results to confirm identity of *T. brucei* *tbat1*<sup>-/-</sup>.

Panel A, neomycin 800 bp PCR product (white arrow head).

Panel B, TbAT1 1400 bp PCR product (white arrow head).

Lane 1, *T. brucei* s427 (wild type with P2 activity).

Lane 2, *tbat1*<sup>-/-</sup> (mutant lacking P2 activity).

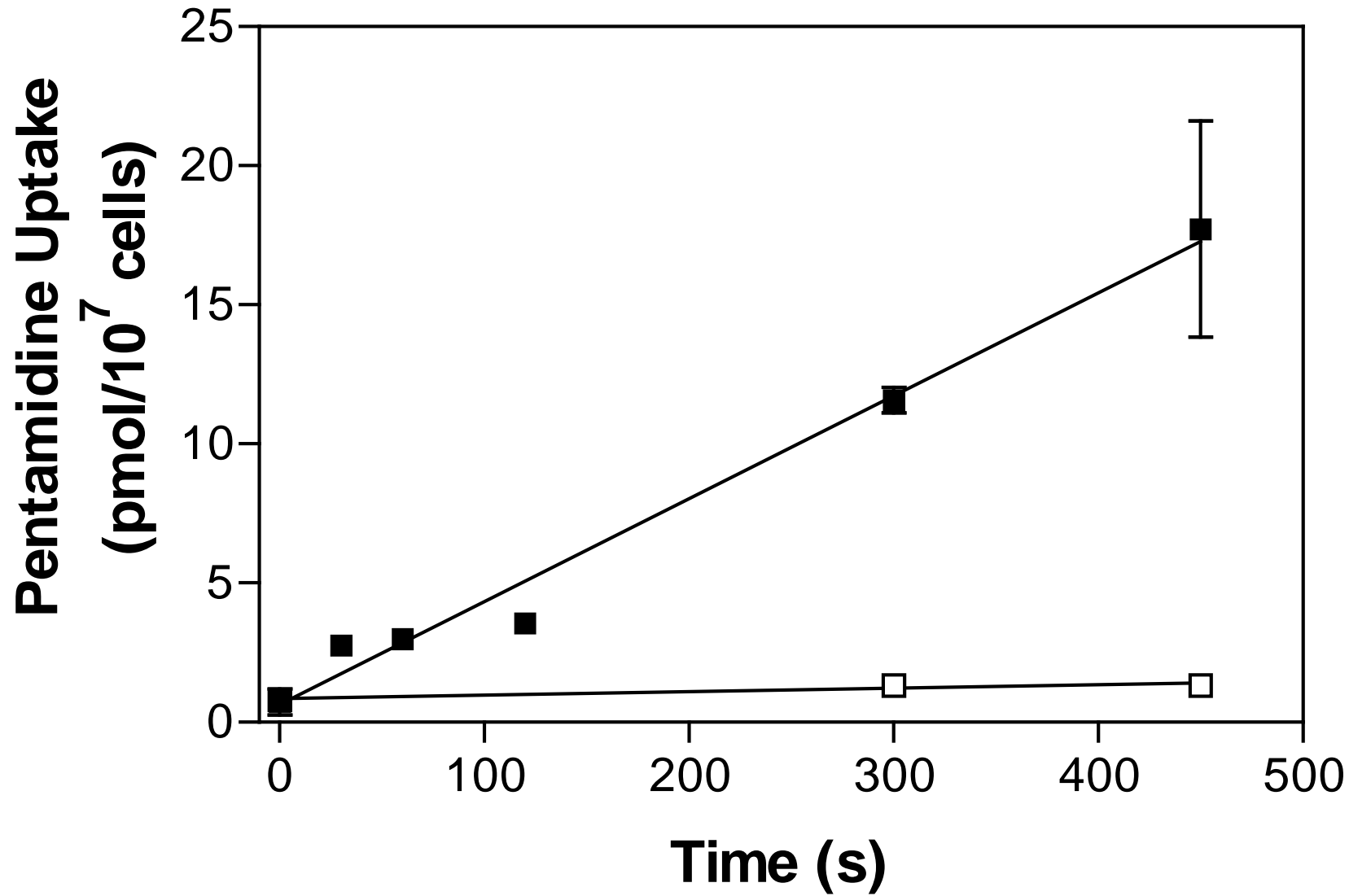
-ve, no template (water) control.

M, 1 Kb plus DNA size marker (Invitrogen, Life technologies, USA).

M', 100 bp DNA size marker (Promega).

Figure S3. Selection for diminazene resistance in the *tbat1*<sup>-/-</sup> cell line cultured under drug pressure. Observed increments in resistance in the *tbat1*<sup>-/-</sup> cell line in comparison to wild type s427 when cultured in increasing drug concentrations over a 6 month period are indicated.

Figure S4. Transport of 1  $\mu\text{M}$  [ $^3\text{H}$ ]-diminazene in the s427WT (A), *tbat1*<sup>-/-</sup> (B), B48 (C) and ABR (D) cell lines. Representative experiments in triplicate are shown (average  $\pm$  SE). Transport rates were calculated by linear regression. Y-axis scales were kept the same for all panels to highlight differences between the cell lines. ■, 1  $\mu\text{M}$  [ $^3\text{H}$ ]-diminazene; □, with the addition of 1 mM unlabelled diminazene.



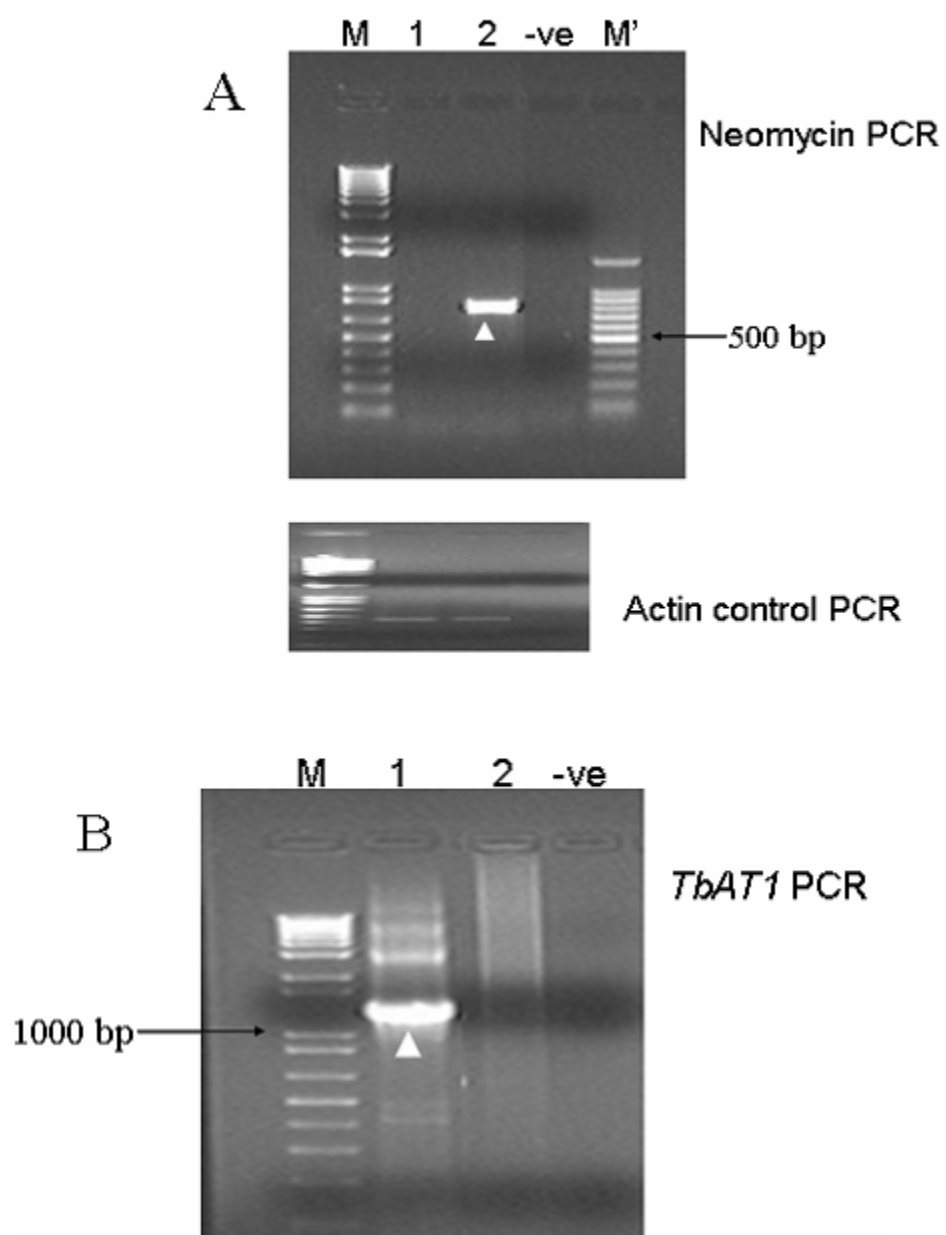


Figure S3

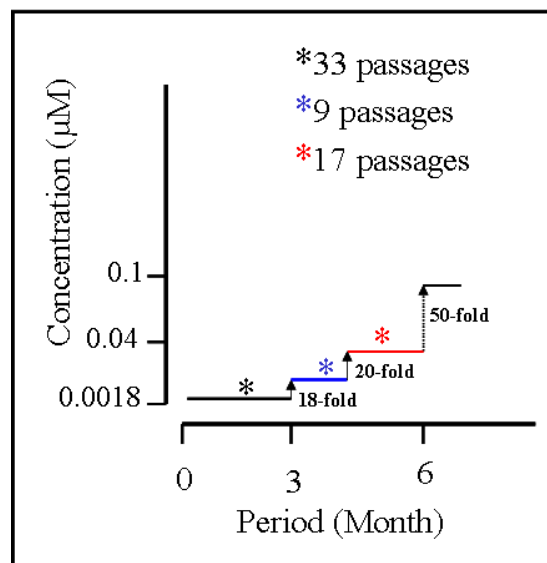


Figure S4

