

***Trypanosoma brucei* (UMP synthase null mutants) are avirulent in mice, but recover virulence upon prolonged culture *in vitro* while retaining pyrimidine auxotrophy**

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Table S1 Growth of WT and DKO parasites cultured in various media

Table S2 Cloning primers

Figure S1 Genotypic analysis of WT, DKO and DKO cell lines isolated from mice

Figure S2 Effect of pyrimidines on the sensitivity of WT cells to pyrazofurin

Supporting information

Table S1 Growth of WT and DKO parasites cultured in various media. The composition of the basal medium is described in Experimental Procedures.

Trypanosome Basal Medium (TBM) Supplements	Doubling time (h)			
	WT plus thymidine	DKO plus thymidine	WT no thymidine	DKO no thymidine
10% FCS / 10% Serum Plus	5.53 ± 0.01 ^a	6.23 ± 0.01 ^a	-	-
10% FCS	6.05 ± 0.01	7.16 ± 0.02	6.11 ± 0.01	7.21 ± 0.01
10% dialysed FCS	7.63 ± 0.01	No growth	7.60 ± 0.01	No growth
10% dialysed FCS / 100 µM uracil	7.35 ± 0.03	7.42 ± 0.04	7.40 ± 0.01	7.61 ± 0.02
10% dialysed FCS / 100 µM uridine	7.35 ± 0.01	17.01 ± 0.25	7.40 ± 0.01	17.18 ± 0.10
10% dialysed FCS / 1,000 µM uridine	7.35 ± 0.02	7.22 ± 0.04	7.35 ± 0.02	7.35 ± 0.01

^aThis medium is commonly referred to as HMI9T, used for routine culture of bloodstream forms of *T. brucei*.

Table S2 Cloning primers

Upper case letters refer to nucleotides corresponding to gene sequences in *T. brucei*; lower case refers to additional sequences used in generating constructs. Restriction endonuclease sites are underlined.

Primer name	Primer sequence
5'UTR-NotI_s	5'ataagaat <u>gcgccgc</u> TACAACATGCGTTTGATCTATCAGT 3'
5'UTR-HindIII/PmeI_as	5'gtttaaacttacggaccgt <u>caagctt</u> TTTGTAAGTGTTCCTTTCACAAC 3'
3'UTR-PmeI/BamHI_s	5'gacggtccgtaagttaa <u>acggatcc</u> GTGGGTCGGAGCTCTATCTTTAAGC 3'
3'UTR-NotI_as	5'ataagtaag <u>gcgccgc</u> TACCCTTCAATGCAGAGGGCACCACAGC 3'

Figure S1. Genotypic analysis of WT, DKO and DKO cell lines isolated from mice.

Southern blot analysis of SacI-digested genomic DNA (~5 µg) WT, DKO and DKO cell lines isolated from 5 different mice using a probe against the 5'-UTR of UMPS.

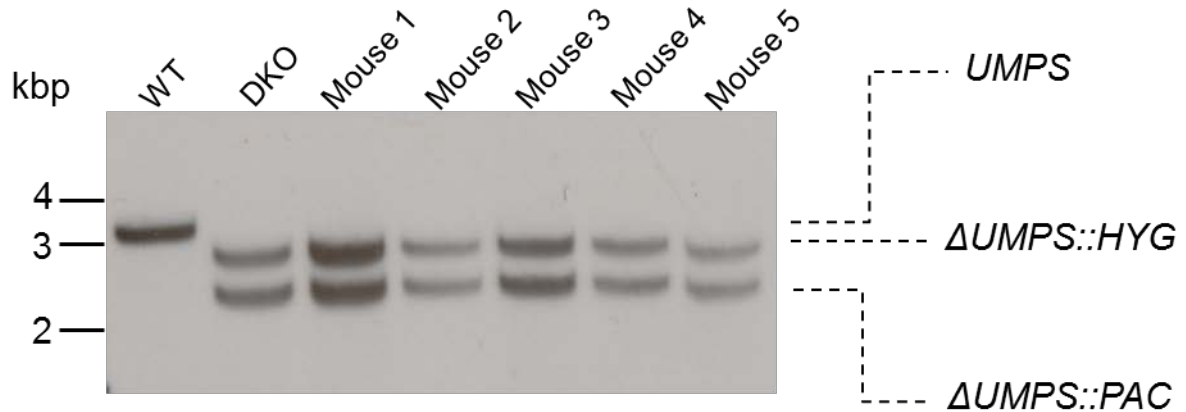


Figure S2 Effect of pyrimidines on the sensitivity of WT cells to pyrazofurin. The EC_{50} values of pyrazofurin against WT cells was determined in TBM^{dFCS} alone (closed circles; $0.42 \pm 0.01 \mu\text{M}$) or in the presence of 100 μM uracil (open squares; $0.44 \pm 0.01 \mu\text{M}$) or 100 μM uridine (closed squares; $0.51 \pm 0.01 \mu\text{M}$) or 100 μM orotate (open circles; $0.43 \pm 0.02 \mu\text{M}$). Results are the means \pm S.E.M. of triplicate measurements.

