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Supplemental Information

Trypanosomatid Deoxyhypusine Synthase Activity Is Dependent on Shared Active-Site Complementation between Pseudoenzyme Paralogs

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Table S1. Role of active site residues, based on published mutational and structural analysis on hDHS (related to Figure 7)**.**

***Tb*DHSc:DHSp**	**hDHS**	**Role and/or mutation effect**
H377A-DHSc:DHSp^	H288-hDHS(Lee et al., 2001, Liao et al., 1998)	Proposed hydride acceptor in (Step 1, Fig. 2)
DHSc:DHSp-H266A^		
DHSc:DHSp-E103A#	E136-hDHS(Umland et al., 2004)	Required for binding of spermidine based on GC7 structure
E166A-DHSc:DHSp#		
D405A-DHSc:DHSp	D316-hDHS(Umland et al., 2004, Lee et al., 2001)	Required for spermidine binding; H-bond with amino group in GC7; possible catalytic base? (Step 2, Fig. 2)
DHSc:DHSp-E104A	E137-hDHS(Umland et al., 2004, Lee et al., 2001)	Required for spermidine binding; mutants are unable to bind spermidine or catalyze spermidine cleavage (Step 2, Fig. 2); NAD binding unaffected
DHSc:DHSp-D218A	D243-hDHS(Umland et al., 2004, Lee et al., 2001)	Prevents spermidine cleavage (Step 2, Fig. 2) due to lack of spermidine binding; NAD binding unaffected

Table S2. Thermal stability of *Tb*DHSc:DHSp WT and mutants (Related to Figure 7**).** The melting temperature (T_m) of each enzyme was measured using a Thermal Shift Assay. All enzymes showed good thermal stability with T_m above 51°C. Errors represent standard deviation for 6 replicates.

Sample	**T_m (°C)**
*Tb*DHSc:DHSp	56.0 ± 0.45
E166A-DHSc:DHSp	51.1 ± 0.66
DHSc:DHSp-E103A	56.1 ± 0.20
DHSc:DHSp-E104A	57.2 ± 0.61
DHSc:DHSp-D218A	57.6 ± 0.58
D405A-DHSc:DHSp	55.5 ± 0.84
DHSc:DHSp-H266A	57.4 ± 1.39
H377A-DHSc:DHSp	55.7 ± 0.41

Figure S1. *Tb*DHSc:DHSp structure (related to Figure 3). Tetramer corresponding to chains A-D as shown in Figure 3. A) *Tb*DHSc:DHSp crystal structure showing the 3 tetrameric molecules in the asymmetric unit. B) Overlay of the 3 tetramers from the asymmetric unit. For each tetramer 5581 atoms were aligned yielding a RMSD < 0.5 Å. Alignments were performed in PyMol using superimpose.