

Size does matter: 18 amino acids at the N-terminal tip of an amino acid transporter in *Leishmania* determine substrate specificity

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Supplementary Information

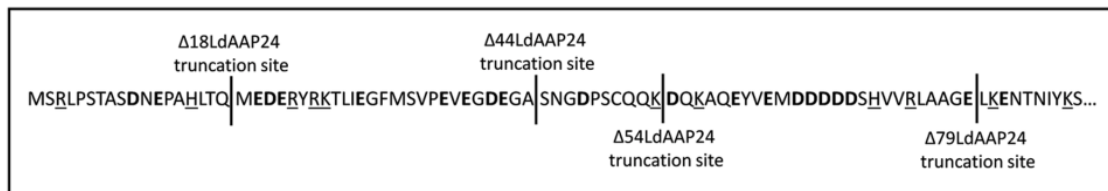


Figure S1. Amino-acid sequence of LdAAP24 N-terminus. Truncation sites for construct preparations are marked.

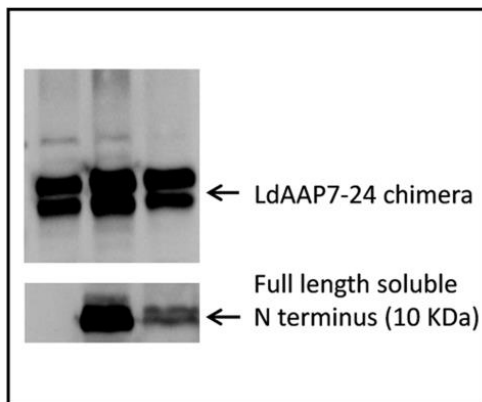


Figure S2. Western blot analysis of Δ LdAAP24 ectopically co-expressing LdAAP7-24 chimera (43 KDa) and the full length N terminus (10 KDa).

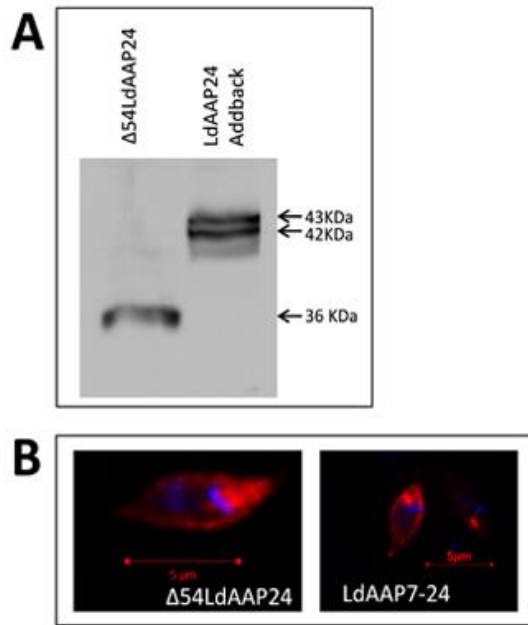


Figure S3. **A.** Western blot analysis on salt-extracted membranes²⁸ of $\Delta LdAAP24$ promastigotes ectopically expressing LdAAP24 (42 and 43 KDa) or $\Delta 54LdAAP24$ (36 KDa). Polyclonal anti-HA antibodies were used as primary antibodies. **B.** Indirect immunofluorescence of LdAAP24-null mutants ($\Delta LdAAP24$) ectopically expressing $\Delta 54LdAAP24$ or LdAAP7-24 chimera. Cells were stained with anti-HA antibodies (red) and the DNA stained with DAPI (blue), the latter stains the nucleus and the kinetoplast. The two fluorescent images were merged.

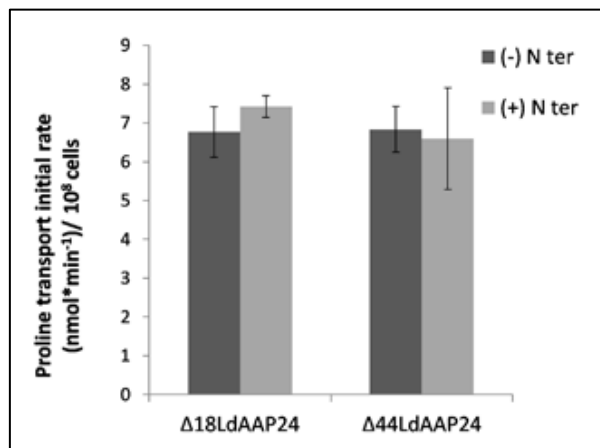


Figure S4. Initial transport rate (3 minutes) of 1 mM ³H L-proline was assayed in LdAAP24-null mutants ectopically expressing $\Delta 18LdAAP24$ / $\Delta 44LdAAP24$ (-N⁰, dark grey) and LdAAP24-null mutants ectopically co-expressing $\Delta 18LdAAP24$ / $\Delta 44LdAAP24$ and the full length N terminus (+N⁰, light grey). Transport was determined at pH 7 and 30 °C. Values represent the mean of at least three independent repeats \pm SD.

Supplementary Table S1. Primer-list.

PCR target	Primer	Restriction enzyme	Plasmid
LdAAP24 N'	Fw: AAAGCTCTCGAGATGTCCAGATTGCCAAGCACC Rv: AAAGCTGGTACCCTACTTACCCAGGCGGTTTCATT TCGATATCAGTGTAGGACTTGCAGATGTTTGTGTTTC	XhoI KpnI	pNUS HnD
LdAAP24	Fw: AAAGCTCTCGAGATGTCCAGATTGCCAAGCACC Rv: AAAGCTGGTACCCTATACACTGATATCGAAATG AACCGCCTGGGTAAGGGACTTGCAGATGTTTGTGTTTC	XhoI KpnI	pNUS HnB
Δ18LdAAP24	Fw: AAAGCTCTCGAGATGGAGGACGAACGTTAC Rv: AAAGCTGGTACCCTATACACTGATATCGAAATG AACCGCCTGGGTAAGGGACTTGCAGATGTTTGTGTTTC	XhoI KpnI	pNUS HnB
Δ44LdAAP24	Fw: AAAGCTCTCGAGATGAGCAATGGAGACCCCTCC Rv: AAAGCTGGTACCCTATACACTGATATCGAAATG AACCGCCTGGGTAAGGGACTTGCAGATGTTTGTGTTTC	XhoI KpnI	pNUS HnB
Δ54LdAAP24	Fw: AAAGCTCTCGAGATGGACCAGAAGGCGCAGG Rv: AAAGCTGGTACCCTATACACTGATATCGAAATG AACCGCCTGGGTAAGGGACTTGCAGATGTTTGTGTTTC	XhoI KpnI	pNUS HnB
Δ79LdAAP24	Fw: ATTATTGGTACCATGACTGTCGTCCGCCT Rv: AAAGCTGGTACCCTATACACTGATATCGAAATG AACCGCCTGGGTAAGGGACTTGCAGATGTTTGTGTTTC	XhoI KpnI	pNUS HnB
LdAAP7-24 chimera	Fw: ACTTATCTCGAGATGAGCGGCGCTAACCACCAC Split Fw: CATCGAGAGGGTCTACTCCATCATGACTGTCGTCCGCCTCGC Split Rv: GCGAGGCGGACGACAGTCATGATGGAGTAGACCCTCTCGATG Rv: AAAGCTGGTACCCTATACACTGATATCGAAATG AACCGCCTGGGTAAGGGACTTGCAGATGTTTGTGTTTC	XhoI KpnI	pNUS HnB