Generation of NIPSNAP-Deficient and Tagged *Leishmania mexicana* Lines for Target Validation Studies

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Callunene is a natural component of heather (*Calluna vulgaris*) nectar that has antiparasitic activity against trypanosomatid species. Recently, we showed that its primary molecular target is mitochondrial protein - NIPSNAP (non-neuronal synaptosomal-associated protein 25). This protein family is characterized by evolutionarily conserved NIPSNAP domains and is implicated in mitochondrial metabolism and, presumably, mitophagy regulation. However, its function in trypanosomatid parasites remains understudied. Trypanosomatids have a single mitochondrion that is crucial for parasite biology and adaptation to the infected host, making it an attractive drug target. In this study, we aim to elucidate the biological role of NIPSNAP in *Leishmania mexicana,* a protistan parasite causing cutaneous leishmaniasis in humans. We generated a Halo-tagged NIPSNAP line and confirmed its mitochondrial localization, consistent with the predicted function. We also ablated the *nipsnap* gene in *L. mexicana* using CRISPR/Cas9 technology. The resulting mutant cell lines enable us to: 1) validate NIPSNAP as a molecular target of callunene and assess its contribution to the compound's antiparasitic activity; 2) investigate the role of NIPSNAP in mitochondrial biology and the function of NIPSNAP in *L. mexicana* development and survival.